ASSESSMENT OF THE IMPACT OF SUNFLOWER OIL EMOLLIENT THERAPY ON NEONATAL SKIN BARRIER FUNCTION AND NUTRITIONAL STATUS IN SARLAHI, NEPAL

by
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Abstract

Background: Over 3 million neonatal deaths occur annually, mostly in developing countries. Neonatal massage with locally available vegetable oils is a low-cost intervention being explored to reduce neonatal morbidity and mortality. Although millions of infants in South Asia are massaged with mustard oil each year, it is unknown whether substituting sunflower seed oil changes any biological mechanisms that may improve neonatal health.

Methods: This was a nested community-based cluster-randomized controlled trial of 1000 neonates (500 preterm (<37 weeks), 500 full term) randomized to promotion of either mustard seed (traditional) or sunflower (improved) oil for full body massage. Skin integrity was assessed using the following measurements: transepidermal water loss (TEWL), skin pH, skin condition (days 1, 3, 7, 14, and 28), and stratum corneum protein concentration (days 1, 7, 14, and 28). Nutritional status was assessed by weight (days 1, 3, 7, 10, 14, 21, and 28) and length (days 1 and 28) measurements. Risk factors associated with skin integrity were evaluated using longitudinal mixed-effects models. Effects of oil group on skin integrity and nutritional status were assessed using longitudinal mixed-effects models, accounting for the clustered design.

Results: Risk factors associated with skin integrity included: sex, birthweight, ethnicity, socioeconomic status, time between measurement and last (i.e. most frequent) massage, temperature, and humidity. In the sunflower oil group, skin pH and TEWL decreased at a faster rate during the neonatal period (0.006 pH units/day and 0.16 $g/m^2/hr$ per day respectively) than in the mustard oil group. Preterm infants' TEWL in the sunflower oil group decreased 0.47 $g/m^2/hr$ per day faster than those in the mustard oil group between days 4 and 28. There was no evidence that skin condition scores, protein concentration, or nutritional status was different between groups.

Conclusions: These data indicate neonatal full body massage using sunflower oil may help to improve skin barrier integrity relative to mustard seed oil. Sunflower oil did not show an improved effect on nutritional status when compared to mustard seed oil. Further research on other possible biological mechanisms that may be related to improved health outcomes in infants massaged with emollients should be considered.

Thesis Advisor: Dr. Luke Mullany

Thesis Committee Members: Dr. Jay Bream, Dr. Joanne Katz, Dr. Edward Lawson

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Table of Contents

List of Tables

List of Figures

Chapter 1 Introduction

Globally, 3.1 million neonatal (≤28 days) deaths occur each year, with 98% of these deaths occurring in developing countries. (Black, Cousens et al. 2010; Lawn, Kerber et al. 2010; Liu, Johnson et al. 2012) An increasing proportion of deaths in children under-five occur during this neonatal period. (Lawn, Cousens et al. 2005; Oestergaard, Inoue et al. 2011; Liu, Johnson et al. 2012) The top three causes of death in these infants are complications from preterm birth (35%), infections (including sepsis, pneumonia, diarrhea, meningitis, and tetanus) (27%), and intrapartum related neonatal deaths, such as birth asphyxia (23%). (Liu, Johnson et al. 2012) Preterm complications also contribute to other causes of death, resulting in half of all neonatal deaths globally as preterm. (Belizan, McClure et al. 2012) In Southeast Asia, 52% of child deaths occur during the neonatal period. (Liu, Johnson et al. 2012)

In order to reach the Millennium Development Goal 4 (MDG4) of reducing child mortality by 2/3 by 2015, the current rate of reduction of under-five mortality must increase 13.5% per year between 2011 and 2015. (Lawn, Kinney et al. 2012) This goal cannot be achieved without a significant reduction in neonatal mortality. Preventing neonatal deaths has not been a traditional focus of child survival or safe motherhood programs. Child survival programs must continue to develop, looking for simple, cost-effective strategies to reduce neonatal mortality in community settings.

One intervention being explored to reduce neonatal mortality is the practice of neonatal oil massage using locally available vegetable oils, which is practiced almost universally throughout Southeast Asia. (Darmstadt and Saha 2002; Mullany, Darmstadt et al. 2005)

In Africa, oil massage is not as commonly practiced as in South Asia however; it is still commonly done in many communities and so could be acceptable as an intervention to improve neonatal mortality and morbidity. (Iweze 1983; Niang 2004; Darmstadt, Hussein et al. 2007; Mrisho, Schellenberg et al. 2008; Waiswa, Nyanzi et al. 2010; Duffy, Ferguson et al. 2012)

Studies have shown that massage with oil is more beneficial than massage alone. (Field, Schanberg et al. 1996) Furthermore, hospital-based studies show that the application of sunflower oil reduces rates of invasive infection and mortality. A study in Egypt of preterm infants <34 weeks comparing the application of sunflower oil to controls showed a significant decrease in the incidence of nosocomial infections and an improvement in skin condition in infants receiving sunflower oil. (Darmstadt, Badrawi et al. 2004) Another study of preterm infants in Bangladesh comparing infants randomized to massage with sunflower oil, Aquaphor, or no emollient, found a 41% reduction in nosocomial infections (Darmstadt, Saha et al. 2005) and a 26% reduction in neonatal mortality rates in the sunflower oil group compared with controls. (Darmstadt, Saha et al. 2008)

Although studies have shown that topical application of vegetable oil may be beneficial to neonates, the choice of oil may be important. A study done using a mouse model showed accelerated skin barrier recovery in mice one hour after application of sunflower oil, with a sustained effect up to five hours. (Darmstadt, Mao-Qiang et al. 2002) However, application of mustard, olive, or soybean oils showed delayed recovery of skin barrier function when compared to untreated or Aquaphor treated skin. Mustard seed oil showed the most detrimental effects, with sustained delay of barrier recovery for up to seven days. One possible reason for this delayed recovery is that mice treated with mustard seed oil showed structural changes in their epidermis, with flattened keratinocytes, apoptotic nuclei and nuclear envelope hypertrophy, and condensed heterochromatin. (Darmstadt, Mao-Qiang et al. 2002)

There are several ways in which emollient therapy with vegetable oil may improve the health of newborns. These include: improving skin integrity and barrier function, changes in the bacteriological profile of the skin, improving the immune response at the systemic and/or epidermal levels, and improving nutritional status through transcutaneous absorption of lipids. (Darmstadt and Dinulos 2000) Despite the interest in neonatal oil massage with locally available vegetable oils as a possible low-cost intervention for improvement of neonatal morbidity and mortality, there has been very little research on the biological mechanisms through which sunflower oil massage may improve neonatal health. This research is important to allow for direct comparison of functional indicators across public health outcomes including neonatal skin infection, clinical signs of sepsis, and mortality. While a mouse model has provided some insights into these emollient-dependent mechanisms, further research in human infants is essential for us to extend our understanding. (Expert Emollients Meeting, Bill and Melinda Gates Foundation, December 14, 2012)

Study Objective:

The objective of this study was to determine the possible mechanistic reasons for improved health outcomes in neonates receiving full body massage using sunflower seed oil relative to mustard seed oil. These activities broadly relate to four domains: improved skin integrity and barrier function, nutritional status, microbial challenge at the skin, and biomarkers of immune responses and are reflected in the following specific aims:

Specific Aims

To determine if sunflower seed oil massage:

1. **improves skin integrity and barrier function.** Specifically, examine if (a) trans-epidermal water loss is decreased, (b) skin pH more rapidly stabilizes during the first week of life, (c) there is a difference in stratum corneum protein content and amount and (d) skin condition scores for dryness, rash, and erythema are lower (improved) among infants in the sunflower oil group
2. **improves nutritional status.** Specifically, examine if (a) weight is higher at days 3, 7, 10, 14, 21, 28, and 180 days after birth, (b) length is greater 28 and 180 days after birth, and (c) serum levels of essential fatty acids are improved among infants in the sunflower oil group
3. **results in changes in bacteriological profile of the skin.** Specifically, examine if overall and organism-specific presence and density of colonization at the axilla, inguinal, and periumbilical region differs between the groups
4. **results in changes in immune response at both the systemic and epidermal innate immunity levels.** Specifically, compare (a) salivary cytokine concentrations over the first two weeks of life using repeat salivary swabs, (b) serum cytokine concentrations on day 14 using heelprick blood samples and (c) epidermal biomarkers (keratin 1,10,11, involucrin, albumin) and cytokine levels (IL-1α, IL-1β, IL-6, IL-8, TNF-α) using a non-invasive skin tape-strip sampling method

This dissertation focuses on the first two specific aims relating to improvements in skin integrity and barrier function and nutritional status. In addition, the analyses presented here represent preliminary work conducted among a subset of the (eventual) sample size. There are six additional chapters included in this dissertation. Chapter 2 provides background information on neonatal massage with and without emollient therapy and its relationship with skin barrier integrity, nutritional status, bacterial colonization, and immune response. Chapter 3 presents an overview of the methodology that was used to address specific aims 1 and 2. Chapter 4 discusses the epidemiology of the skin integrity measurements and the possible risk factors associated with them in this population. Chapter 5 addresses whether full body oil massage using sunflower seed oil improves skin integrity and barrier function relative to massage with mustard seed oil. Chapter 6 investigates whether massage with sunflower oil improves nutritional status when compared with massage using mustard seed oil. Chapter 7 is the final chapter and provides a discussion of the results presented in Chapters 4 through 6, strengths and limitations of the study, as well as recommendations for further research.

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Chapter 2 Background

Introduction

Globally, 3.1 million neonatal (≤28 days) deaths occur each year, with 98% of these deaths occurring in developing countries based on estimates from the year 2010. (Black, Cousens et al. 2010; Lawn, Kerber et al. 2010; Liu, Johnson et al. 2012) This accounts for more than 40% of all deaths in children under-five years, an increase in the proportion of child deaths that occurred during the neonatal period according to year 2000 estimates. (Lawn, Cousens et al. 2005; Oestergaard, Inoue et al. 2011; Liu, Johnson et al. 2012) The top three causes of death in these infants are complications from preterm birth (35%), infections (including sepsis, pneumonia, diarrhea, meningitis, and tetanus) (27%), and intrapartum related neonatal deaths, such as birth asphyxia (23%). (Liu, Johnson et al. 2012) However, in settings with high neonatal mortality (NMR), the proportion of deaths due to infections is about 50%. (Lawn, Cousens et al. 2005; Blencowe and Cousens 2013) Preterm complications also contribute to other causes of death, resulting in half of all neonatal deaths globally as preterm. (Belizan, McClure et al. 2012) Preterm infants have almost a seven-fold greater risk of neonatal mortality than term infants. (Katz, Lee et al. 2013) Sub-Saharan Africa and Southern Asia account for 52% of all live births, but 78% of neonatal deaths. (Oestergaard, Inoue et al. 2011) In addition, 60% of preterm births occur in Africa and South Asia. (WHO 2012)

At least half of all neonatal deaths occur in the first 24 hours and three-quarters of all neonatal deaths occur during the first week of life, with many of these deaths going unrecorded. (Lawn, Cousens et al. 2005; Belizan, McClure et al. 2012) Preventing neonatal deaths has not been a traditional focus of child survival or safe motherhood programs. Child survival programs have mostly focused on interventions to combat diseases such as diarrhea, pneumonia, malaria, and vaccine preventable diseases that primarily affect children who are past their neonatal period. As a result, child mortality (2 months to 5 years) saw a reduction of one-third from 1989-2000, while NMR only saw a one-fourth reduction. (Lawn, Cousens et al. 2005) The average annual rate of reduction of NMR from 2000 to 2010 accelerated 2.1% per year when compared with the 1990s, however this was slower than both child mortality and maternal mortality. (Hill, You et al. 2012) Official development assistance for maternal, newborn, and child health doubled from 2003 to 2008, but by 2008 only 6% of aid mentioned newborns and only 0.1% exclusively targeted newborn care. (Lawn, Kinney et al. 2012)

In Southeast Asia, 52% of child deaths occur during the neonatal period and in Nepal it is estimated that in 2010, there were 20,049 neonatal deaths, with 58% of under-five deaths occurring during the neonatal period. (Liu, Johnson et al. 2012) Poverty is associated with an increased risk of neonatal death and considerable income inequalities exist in Nepal, leading to large health disparities. For instance, Nepal would show a 43% reduction in NMR if the NMR for the entire population were the same as for the richest 20% of Nepalese. (Lawn, Cousens et al. 2005) The poorest quintile is 65% more likely to have a neonatal death than the wealthiest quintile. (Pradhan, Upreti et al. 2012) These numbers are most likely underestimates, as studies have shown that in poor settings in Southeast Asia, neonatal deaths are greatly underestimated. (Bang, Reddy et al. 2002) However, between 2000 and 2010 the countries with the greatest overall reductions in NMR in Southern Asia were Iran, Bangladesh, and Nepal. (Lawn, Kinney et al. 2012) Nepal's NMR has decreased from 50 per 1000 live births in 1996 to 27 per 1000 live births in 2011. (Ban, Tuladhar et al. 2012; United Nations Children's Fund 2013)

In order to reach the Millennium Development Goal 4 (MDG4) of reducing child mortality by 2/3 by 2015, the current rate of reduction of under-five mortality must increase 13.5% per year between 2011 and 2015. (Lawn, Kinney et al. 2012) This goal cannot be achieved without a significant reduction in neonatal mortality. Child survival programs must continue to develop, looking for simple, cost-effective strategies to reduce neonatal mortality in community settings. While Nepal is one of the low-income countries on track to meet MDG4, many inequities exist. (Lozano, Wang et al. 2011) Urban children have better chances of survival than rural children and ethnic groups such as the *Dalits* and Terai *Madhesis* have higher relative levels of neonatal mortality. (Nguyen, Jimenez-Soto et al. 2013) NMR is nearly double in rural and mountainous regions in Nepal and between 1996 and 2006 the inequity in NMR between advantaged and disadvantaged caste/ethnic groups increased. (Pradhan, Upreti et al. 2012)

One intervention being explored to reduce neonatal mortality is the practice of neonatal oil massage using locally available vegetable oils, which is practiced almost universally throughout Southeast Asia. A study in Bangladesh found that >96% of neonates regardless of socioeconomic factors or place of residence, received full body massage, with mustard seed oil as the most commonly used oil. The perceived benefits from the mothers included preventing infections, keeping the infant warm, improving skin condition, and improving overall health. (Darmstadt and Saha 2002) Another study in Sarlahi district in rural Nepal, found that 99% of newborns received oil massage at least once in the first two weeks after birth, with mustard oil again as the oil of choice. This was practiced for all ethnicities, races, and cultures, and did not differ by socioeconomic status. The perceived benefits were similar to those cited by mothers in Bangladesh: the massage promotes strength, maintains health, and provides warmth. (Mullany, Darmstadt et al. 2005)

Studies done in Africa show that while oil massage is not as common a practice as in South Asia, it is still commonly done in many communities and so could be acceptable as an intervention to improve neonatal mortality and morbidity. (Duffy, Ferguson et al. 2012) In Nigeria after birth, infants are commonly rubbed with palm oil or kernel oil before their first bath in order to remove impurities and after the bath in order to increase limb strength. (Iweze 1983) In Tanzania, after birth, sesame or other cooking oil is often applied to the body to soften the skin and warm the infant. (Mrisho, Schellenberg et al. 2008) Cooking oil is commonly applied to infants' skin in Uganda in order to "make the skin strong" (Waiswa, Nyanzi et al. 2010) and in Senegal infants are often massaged soon after birth and then daily for the first one or two months with cooking oil in order to "massage all of the organs, move them in all directions, and give them shape". (Niang 2004) In addition, a study on neonatal home care in rural Egypt found that use of a skin care product was common, mostly in the first or second day of life and that most products were applied one to three times per day, with the most common reasons being to prevent skin infection and improve skin condition. Most mothers also said they would be willing to apply a prescription skin oil or emollient to prevent infections. (Darmstadt, Hussein et al. 2007)

Many studies have shown that massage with oil is more beneficial than massage alone. A study of infants massaged with Johnson and Johnson baby oil versus infants massaged without oil showed those massaged with oil to show fewer stress behaviors, lower salivary cortisol levels, and increased vagal activity. (Field, Schanberg et al. 1996) Another study of preterm neonates admitted to a neonatal intensive care unit in Iran who were randomized to moderate pressure massage alone and the same massage using sunflower oil, showed greater mean weight gain at one and two months in neonates in the group massaged with sunflower oil. (Fallah, Akhavan Karbasi et al. 2013) A study of preterm neonates less than 1800g in a neonatal intensive care unit in India comparing infants massaged with sunflower oil with standard care to those only receiving standard care found those infants receiving oil massage to have greater weight gain at 28 days and less weight loss at seven days than those who were not receiving oil massage. (Kumar, Upadhyay et al. 2013) Another study in India with preterm neonates randomized to oil massage, massage without oil, and no massage, also showed oil massage may have the ability to improve weight gain in very low birthweight preterm infants. (Arora, Kumar et al. 2005)

In addition, hospital-based studies have shown that the application of sunflower oil reduces rates of invasive infection and mortality. A study in Egypt of preterm infants <34 weeks comparing the application of sunflower oil to controls showed a significant decrease in the incidence of nosocomial infections and an improvement in skin condition in infants receiving sunflower oil. (Darmstadt, Badrawi et al. 2004) Another study of preterm infants in Bangladesh comparing infants randomized to massage with sunflower oil, Aquaphor, or no emollient, found a 41% reduction in nosocomial infections (Darmstadt, Saha et al. 2005) and a 26% reduction in neonatal mortality rates in the sunflower oil group compared with controls. (Darmstadt, Saha et al. 2008) Many families from this same cohort perceived topical therapy with sunflower oil or Aquaphor to be better than mustard oil. (Ahmed, Saha et al. 2007)

Although studies have shown that topical application of vegetable oil may be beneficial to neonates, the choice of oil may be important. A study done using a mouse model showed accelerated skin barrier recovery in mice one hour after application of sunflower oil, with a sustained effect up to five hours. (Darmstadt, Mao-Qiang et al. 2002) However, application of mustard, olive, or soybean oils showed delayed recovery of skin barrier function when compared to untreated or Aquaphor treated skin. Mustard seed oil showed the most detrimental effects, with sustained delay of barrier recovery for up to seven days. One possible reason for this delayed recovery is that mice treated with mustard seed oil showed structural changes in their epidermis, with flattened keratinocytes, apoptotic nuclei and nuclear envelope hypertrophy, and condensed heterochromatin. (Darmstadt, Mao-Qiang et al. 2002) In addition, a study looking at differences in application of olive oil and sunflower oil on adult forearms found that after 4 weeks, olive oil caused a significant reduction in skin integrity and induced mild erythema, whereas the sunflower oil preserved skin integrity and improved hydration. (Danby, AlEnezi et al. 2013)

There are several ways in which emollient therapy with vegetable oil may improve the health of newborns. These include: improving skin barrier integrity and function, changes in the bacteriological profile of the skin, improving the immune response at the systemic and/or epidermal levels, and improving nutritional status through transcutaneous absorption of lipids. Table 2-1 describes some of the potential benefits of emollient therapy and the possible biological bases for those effects.

Skin Structure and Function

Newborn skin is an important organ in protecting the neonate. It serves as the first line of defense between the infant and the environment, the stratum corneum being where most of this important barrier function occurs. The skin is well developed and fully functional at birth in full term infants, with a thick epidermis and well-formed stratum corneum layers, despite being exposed to water and amniotic fluid for nine months. (Cunico, Maibach et al. 1977; Yosipovitch, Maayan-Metzger et al. 2000)

As an infant's first line of defense, the skin serves many important functions. These include: moderating fluctuations in transepidermal water loss (TEWL) and maintaining electrolyte homeostasis, thermoregulation and minimizing caloric losses, antimicrobial defense, protection from environmental toxins, protection from ultraviolet (UV) radiation, and tactile stimulation. The stratum corneum contains the epidermal permeability barrier and is mainly composed of hydrophobic lipids (free fatty acids, cholesterol, and ceramides). These three stratum corneum lipids are required for permeability homeostasis and a mixture of physiological lipids cholesterol, ceramide, palmitate and linoleate (ratio 3:1:1:1) was shown to be optimum for barrier repair. (Mao-Qiang, Feingold et al. 1996)

This skin barrier is formed in utero during the third trimester, with the stratum corneum starting to develop at about 24 weeks gestation. After 24 weeks the number of epidermal cell layers and the epidermal thickness steadily increase and are well developed at around 34 weeks. (Cartlidge 2000) Therefore, preterm infants before this time show immature barrier function, including: structural immaturity (i.e. the stratum corneum and epidermis are thinner leading to increased susceptibility to shear forces), an underdeveloped stratum corneum epidermal permeability barrier (leading to increased TEWL, loss of heat, increased caloric demands, an increase in potential for environmental toxin absorption, and compromised antimicrobial defense), slower formation of the antibacterial acid mantel, incomplete development of vernix (and subsequent effects), and increased UV susceptibility. (Evans and Rutter 1986; Cartlidge 2000; Darmstadt and Dinulos 2000; Rutter 2000) Epidermal barrier properties such as increased hydration and water binding undergo progressive changes during a healthy full term infant's first month of life as it adapts to the dry extrauterine environment. (Visscher, Chatterjee et al. 2000) Neonatal skin is always adjusting to its extrauterine environment when comparing parameters such as skin thickness, skin pH, TEWL, lipid content, and stratum corneum hydration to adult skin, which is in a steady state. This suggests that a balance between different parameters may be important for barrier function. (Chiou and Blume-Peytavi 2004; Fluhr, Darlenski et al. 2012; King, Balaji et al. 2013) In addition, water handling behaviors and levels of stratum corneum water binding amino acids continue to develop during the first year of life. (Nikolovski, Stamatas et al. 2008)

The skin's antimicrobial defense arises from factors inherent to the stratum corneum (xeric environment, acidic pH, antimicrobial lipids, and continued shedding of corneocytes), cationic antimicrobial peptides, cytokines (IL1-α, IL1-β, TNF-α, IL-10, and IL-12), granzyme B, Fas ligand, nitric oxide, and resident cutaneous flora. (Darmstadt and Dinulos 2000) Corneocytes and the extracellular matrix mediate this defense. Secreted hydrophobic lipids are organized into lamellar bilayers that are critical to permeability barrier and microbial function. (Elias 2007) The multiple functions of the stratum corneum and their relationships are shown in Figure 2-1.

Neonates, especially those who are preterm, are at high risk for infection. In preterm infants, 50% of neonatal mortality is caused by septicemia. (Bartlett, Paz de Bocaletti et al. 1991; Stoll 1997) This high risk of infection could be due in part to their epidermal barrier immaturity (including fewer stratum corneum layers, poorly formed lipid layers, and increased permeability), developmental defects in the systemic immune function, and disordered cutaneous immunoregulatory function because of barrier distress or decreased cationic antimicrobial peptide expression. (Nickoloff and Naidu 1994; Nishijima, Tokura et al. 1997) Three-fourths of all neonatal deaths occur during the first week of life when the epidermal barrier is least mature. (Lawn, Cousens et al. 2005)

Maturation of the skin barrier in premature infants is usually complete in 2-4 weeks (Harpin and Rutter 1983), but may take as long as 9 weeks post neonatal age in very premature infants. (Harpin and Rutter 1983; Agren, Sjörs et al. 1998; Kalia, Nonato et al. 1998; Nonato, Lund et al. 2000) In addition, the permeability of premature skin is much higher than that of adult skin, which can lead to absorption of foreign substances and pathogens. (Nachman and Esterly 1971; Harpin and Rutter 1983)

Premature and low birthweight infants have increased skin permeability (Cartlidge and Rutter 1992) and are prone to skin breakdown. (Malloy and Perez-Woods 1991) The dermis is lacking in structural proteins, leading to poor mechanical properties. (Eichenfield and Hardaway 1999) Stress to the epidermal layer caused by handling or massage could exacerbate this breakdown. (Darmstadt and Dinulos 2000) Several studies have shown emollient therapy to improve overall skin condition in neonates. Very low birthweight infants treated with Aquaphor had improved skin scores when compared with untreated infants. (Pabst, Starr et al. 1999) Other studies of premature infants found infants treated with ointment had a decreased severity of dermatitis. (Lane and Drost 1993; Nopper, Horii et al. 1996)

Another important function of the skin barrier is to protect against transepidermal water loss (TEWL), a direct function of gestational age. (Hoath and Maibach 2003) TEWL in full term infants is very low at birth, equal to or lower than adult values, which indicates a highly effective skin barrier. (Cunico, Maibach et al. 1977; Yosipovitch, Maayan-Metzger et al. 2000) There is an exponential relationship between TEWL and gestational age continuing during the four weeks after birth. (Hammarlund and Sedin 1979) Very low birthweight preterm infants have very high TEWL when measured at birth, ranging from 50 to 70 $g/m^2/hr$. (Hammarlund and Sedin 1979) A reference dataset of over 1000 term neonates had a mean TEWL of 7.06 $g/m^2/hr$. (Kelleher, O'Carroll et al. 2013) This difference decreases with age of the infants and after two weeks, values of TEWL in preterm infants are similar to those in term infants. (Harpin and Rutter 1983) However, in very preterm infants (24-25 weeks gestational age) TEWL has been shown to remain higher after 4 weeks. (Agren, Sjörs et al. 1998) In addition to gestational age, TEWL is also dependent on the site of measurement, ambient humidity, temperature, activity, and nutritional status at birth. (Oberg, Hammarlund et al. 1981; Hammarlund, Sedin et al. 1982; Hammarlund, Sedin et al. 1983) TEWL decreased at a slower rate in infants in an environment with a higher relative humidity, indicating that the level of relative humidity influences skin barrier development with more rapid barrier formation in infants at a lower relative humidity. (Agren, Sjörs et al. 2006) TEWL also increased when temperature was above 37.1°C. (Sedin, Hammarlund et al. 1983) This is important in an environment such as the Terai region of Nepal, which experiences very high temperatures and levels of humidity, particularly during monsoon season. Trauma of the skin can also cause an increase in transepidermal water loss. (Harpin and Rutter 1983) High transepidermal water loss can lead to problems in temperature control due to high evaporative heat, which may lead to hypothermia. (Rutter 1988) TEWL has been shown to decrease in infants treated with emollient therapy. A study of preterm very low birthweight infants randomized to topical coconut application versus no treatment showed a significantly lower TEWL at each point of measurement in the coconut oil group. TEWL was reduced by as much as 46%. (Nangia, Paul et al. 2008)

The acidic pH of the skin is another mode of protection. This acidic surface of the skin is important in the formation and integrity of the stratum corneum. It allows the effective functioning of enzymes that are involved in lipid metabolism, bilayer structure, ceramide synthesis, and desquamatization. (Rippke, Schreiner et al. 2002; Schmid-Wendtner and

Korting 2006) Applying acidic treatments has been proposed for the treatment of inflammation and the normalization of stratum corneum structure and function. (Hachem, Roelandt et al. 2010) In order for the skin to maintain bacteriological, chemical and mechanical resistance the 'acid mantle' of the skin must have an optimal pH (Schmid-Wendtner and Korting 2006). After birth, skin pH is elevated in full term and preterm neonates compared with adults and older children and decreases in the first few weeks, most significantly during the first 4 days. (Green, Carol et al. 1968; Fluhr, Pfisterer et al. 2000; Visscher, Chatterjee et al. 2000; Hoeger and Enzmann 2002) The mean skin pH value of full term neonates within the first 10 hours after birth from six different body sites was 7.08 compared to a mean of 5.7 in adults. (Yosipovitch, Maayan-Metzger et al. 2000; Hoeger and Enzmann 2002) At days 1, 2, and 3 after birth there was no difference in skin pH levels at different sites. (Yosipovitch, Maayan-Metzger et al. 2000) Some studies have suggested development of the acid mantle is not correlated with gestational age, with development in preterm neonates shown to be very close to that of full term neonates. (Green, Carol et al. 1968) However, other studies have shown that for very preterm infants, skin pH tends to remain higher for a longer period of time. (Fox, Nelson et al. 1998)

This optimal pH level is essential for epidermal barrier maturation and repair processes, since important enzymes such as beta-glucoerebroisdase and acidic sphingomyelinase that assist in the processing of lipids that make up the stratum corneum perform optimally only at a certain pH level. (Mauro, Grayson et al. 1998) Degradation of these enzymes occurs as skin pH levels increase. (Hachem, Crumrine et al. 2003; Hachem, Man et al. 2005) A study looking at skin care practices in a Neonatal Intensive Care Unit (NICU) found that using skin wipes with emollient cleansers resulted in significantly lower skin pH levels compared with using a cloth and water. (Visscher, Odio et al. 2009)

Stratum corneum cohesion is reduced with delays in appropriate acidification of the skin, potentially leading to increased risk of micro-injury from mechanical stress (i.e. massage). (Fluhr, Kao et al. 2001) Furthermore, acidic skin pH can enhance epidermal innate immune function by inhibiting colonization of some pathogens and maintaining bacterial homeostasis. (Puhvel, Reisner et al. 1975; Aly, Shirley et al. 1978; Fluhr, Kao et al. 2001; Elias 2007) In addition, if different emollients result in different pH levels of the skin, the changes in pH dynamics may influence the type and density of pathogens colonizing the skin. (Elias 2007) A hospital-based study in Bangladesh showed that preterm neonates treated with sunflower oil and Aquaphor had similar skin flora between groups, however in the neonates treated with sunflower oil, there were fewer pathogens in the blood. (Darmstadt, Saha et al. 2007)

Bacterial Colonization

Bacterial colonization occurs as soon as the infant enters the external world. Infants born by cesarean section are sterile if the amniotic membranes were not ruptured before labor, while infants born vaginally become colonized during their passage through the birth canal. (Sarkany and Gaylarde 1967)

Three types of skin flora have been described. The first is resident bacterial flora, which is relatively stable in number and composition. The second is transient flora. These are bacteria that are derived from exogenous sources and vary in types and amounts. (Price 1938) A third type of skin flora that has been described are nomads or associate flora, which reside and multiply on the skin for only a short amount of time and depend on resident flora for their proliferation. (Brock 1966; Somerville-Millar and Noble 1974) Table 2-2 shows a list of common skin flora colonizing the newborn skin.

Staphylococcus epidermidis, the most common vaginal organism before birth, rapidly colonizes the skin, and is therefore the most common organism on the skin of most neonates. (Sarkany and Gaylarde 1967) After the first few weeks of life, under normal conditions, the skin flora of the infant is nearly the same as that of an adult. In infants who are not immune-compromised and have normal epidermal barriers, these skin-colonizing flora are not virulent, are stable in number, and non-pathogenic. However, in very low birthweight infants whose skin and immune system are not fully developed, *Staphylococcus epidermidis* is the most common cause of sepsis. (Sidbury and Darmstadt 2002) A hospital-based study looking at bacterial colonization of the skin in neonates in Nepal found coagulase-negative Staphylococci (42.1%) *S. aureus* (29.4%) and *E. coli* (24.0%) to be the most common organisms colonizing the skin. (Mullany, Khatry et al. 2008) Another study done in a neonatal unit in a tertiary care teaching hospital in North India found that out of the culture proven cases, the three most common pathogens were Pseudomonas (60%), *S. aureus* (13%) and *E. coli* (13%). They also found that 83.3%, 30.5%, and 80.6% of cases occurred in low birthweight, very low birthweight, and preterm infants respectively. (Chacko and Sohi 2005) Other studies investigating antibiotic susceptibility of pathogens causing neonatal sepsis have found frequent multi-drug resistance. (Tallur, Kasturi et al. 2000; Jyothi, Basavaraj et al. 2013)

Stress to the stratum corneum, such as massage, may cause tiny fissures helping to introduce pathogenic bacteria into the blood stream. (Darmstadt, Saha et al. 2003) For instance, a break in the stratum corneum is required for streptococcal infection to occur. (Leyden, Stewart et al. 1980) In patients with dermatitis, *S. aureus* colonization is directly related to changes in the epidermis (Leyden, Marples et al. 1974; Nilsson, Henning et al. 1986) and the presence of a single suture can increase the infectiousness of *S. aureus* by a factor of 10,000. (Elek 1956) A study of the incidence and etiology of community acquired neonatal bacteremia in Bangladesh found *S. aureus* to be the most common pathogen (1/3 of all pathogens identified). (Darmstadt, Saha et al. 2009) A review of hospital-acquired neonatal infections in developing countries found that gram-negative bacteria and *S. aureus* are the major causes of neonatal sepsis. In South Asia gram-negative bacteria and *S. aureus* accounted for 63.4% and 20.2% of neonatal sepsis respectively. This pattern was observed even in early-onset sepsis (within the first week), which supports the hypothesis that in developing countries the majority of early-onset infections in hospital-born infants may be hospital-acquired and not maternally acquired. (Zaidi, Huskins et al. 2005) A review looking at the etiology of community-acquired neonatal sepsis showed similar patterns of infection with 60.8% and 9.9% of infections in South Asia caused by gram-negative bacteria and *S. aureus* respectively. Again, these same pathogens are the main causes of sepsis during the first week of life, indicating that the infections may be environmentally rather than maternally acquired in this setting. (Zaidi, Thaver et al. 2009) Another review examining the etiology of community-acquired neonatal sepsis further supports this theory, finding a predominance of gram-negative organisms and a similar distribution of pathogens in neonates with early-onset and late-onset (8-59 days of life) sepsis. (Waters, Jawad et al. 2011) Topical emollient therapy could help protect against environmentally acquired infections as it has been shown to reduce bacterial colonization in premature infants (Nopper, Horii et al. 1996) and has been described as an area needed for further research for prevention of neonatal infections. (Osrin, Vergnano et al. 2004)

Structural and Immunological Biomarkers

The stratum corneum is the outermost layer of the skin and is made of nuclear skin cells called corneocytes, forming corneocyte envelopes. The cells of the stratum corneum are made up of many different proteins. Corneodesmosomes are macromolecular glycoprotein complexes within the corneocyte envelope that hold the cells in the stratum corneum together. (Serre, Mils et al. 1991) Small proline-rich (SPRR) proteins help in the strength and flexibility of the corneocyte envelopes. (Cabral, Voskamp et al. 2001)

Involucrin and keratin are structural proteins that help to make up the stratum corneum. Involucrin is a stratum corneum precursor and a component of the keratinocyte cross-linked envelope. (Murphy, Flynn et al. 1984) In stratum corneum positive for involucrin, the cornified envelopes are fragile and have been linked with inflammation and weak barrier function. (Hirao, Terui et al. 2003) In addition, early expression of involucrin has been associated with barrier disruption. (Ekanayake–Mudiyanselage, Aschauer et al. 1998) A reduction in keratin 1,10,11 has been linked with higher skin dryness and chronic hyperproliferation. (Engelke, Jensen et al. 1997)

Albumin is the main protein found in plasma and binds unsaturated fatty acids and calcium. It may also be involved in transport. (Hasse, Kothari et al. 2005) It helps to protect against H_2O_2 oxidation that may occur in the epidermis (Rokos, Moore et al. 2004) and has a positive correlation with transepidermal water loss and a negative correlation with skin hydration. (Yamane, Moriyama et al. 2009)

A study examining biomarkers of innate immunity found keratin 1,10,11 was decreased and involucrin was increased in all infants when compared with adult levels. Involucrin was found to be higher in preterm than full term infants and adults and to be higher in full term infants when compared with adults. Albumin levels were also found to be higher in preterm infants than in full term infants or adults. In addition when comparing epidermal cytokine levels, all infants had elevated IL1-α and reduced TNF-α compared with adults. Preterm infants also had significantly higher IL-6, IL-8, and albumin compared with full term infants and adults. (Narendran, Visscher et al. 2010) Another study examining host defense proteins on neonatal skin compared to adults' skin, found total protein was 2.8 times higher in adults compared to newborns. However, host defense proteins lysozyme and lactoferrin were detected in all samples with lysozyme concentrations five-fold higher in neonates and lysozyme enzyme activity four-fold higher in neonates than in adults, demonstrating that newborn skin stratum corneum has classic host defense proteins. (Walker, Akinbi et al. 2008) These host defense proteins are also present in the vernix caseosa and amniotic fluid in addition to the skin of healthy newborns, indicating effective innate immunity during fetal and neonatal life. (Marchini, Lindow et al. 2002; Yoshio, Tollin et al. 2003; Akinbi, Narendran et al. 2004; Tollin, Bergsson et al. 2005; Walker, Akinbi et al. 2008)

There are several suggested functions of antimicrobial peptides that reside on the skin, such as: 1) antimicrobial action, 2) recruitment of inflammatory cells, 3) wound repair, 4) apoptosis, and 5) stimulate release of pro- and anti-inflammatory factors (including cytokine release). Peptides HBD1 and HBD2 show bactericidal activity against gram-negative bacteria and HBD3 is effective against gram-positive *S. aureus*. In addition, LL-37 and HBD2 show bactericidal activity against Group B Streptococcus. (Yoshio, Lagercrantz et al. 2004)

Serum cytokine as well as epidermal cytokine levels are associated with damage and stress to the stratum corneum, with changes in levels of pro-inflammatory cytokines such as IL1-α, IL1-β, IL-6, IL-8, and TNF-α. Cytokines are small proteins that are released by cells in response to an activating stimulus. Cytokines such as IL1-α, IL1-β, IL-6, IL-8, and TNF-α are mainly produced by macrophages and promote inflammation and fever as well as further activation of the immune response. (Murphy, Travers et al. 2008)

Levels of IL-6 increase after stratum corneum barrier damage and application of IL-6 increased barrier repair in animals. (Wang, Schunck et al. 2003) When barrier function is compromised, epidermal keratinocytes become activated quickly, resulting in increased keratinocyte proliferation and production of specific cytokines (TNF-α, IL-8, IL-10, INF-Y) and adhesion molecule mRNA and proteins from both the epidermis and dermis. (Nickoloff and Naidu 1994) In addition, patients with certain chronic inflammatory diseases show elevated levels of IL1-β, TNF-α, and IL-10 in the skin, when compared with healthy controls. (van der Zee, de Ruiter et al. 2011)

Preterm infants show higher levels of pro-inflammatory cytokines in both the serum (Skogstrand, Hougaard et al. 2008; Matoba, Yu et al. 2009) and the epidermal layer (Narendran, Visscher et al. 2010) and are at higher risk of infection than full term infants as a consequence of this inflammatory process. (Yoon, Romero et al. 2000; Krueger, Nauck et al. 2001) A hospital-based study in Bangladesh showed that preterm neonates treated with sunflower oil applications to the skin reduced the number of pathogens in the blood, when compared with neonates treated with Aquaphor and untreated controls. (Darmstadt, Saha et al. 2007) These changes may be expressed by differences between cytokine levels and profile over the first week of life in groups of infants massaged with sunflower oil compared with mustard seed oil.

Another medium in which to measure cytokine levels is saliva. Salivary measurements of immune markers have been internally validated, however serum-saliva correlations are modest. (Granger, Granger et al. 2006) Taking cytokine measurements from several different sources (serum, saliva, the epidermis) is more likely to capture whether immune function is different in each group than taking measurements from only one source.

Nutrition

Improved nutritional status is another way topical emollient application may be beneficial to neonates. Nutritional deficiencies in infants can result from many sources such as inadequate diet, impaired absorption, high metabolic demands due to relatively large surface areas and rapid tissue growth, and defective metabolism of nutrients. Premature infants may be even more at risk because of a lack of nutrient stores. (Darmstadt 1998)

Low birthweight infants (<2500g) account for 14% of all births and a disproportionate amount of neonatal deaths (60-80%). (Bang, Reddy et al. 2002) About half of the 18 million low birthweight births each year occur in South Asia. (United Nations Children's Fund 2009) This is a prevalent condition in Nepal, with 18% of all infants being low birthweight. (United Nations Children's Fund 2013) In our study location in Sarlahi, ~30% of infants are low birthweight. (Tielsch, Darmstadt et al. 2007) In addition, children grow up to be chronically malnourished with 16.2% of children under-five in Nepal having severe chronic malnutrition and 40.5% of children under-five having moderate or severe malnutrition in 2010. (Nepal Ministry of Health and Population 2011)

Studies have demonstrated that application of vegetable oils to the skin may improve nutrition in neonates and increase rates of weight and length gain. In one study, infants were randomly assigned to receive massage with herbal oil, sesame oil, mustard oil, or mineral oil for 4 weeks. Massage improved weight, length, mid-arm, and mid-leg circumference when compared with non-massaged in all groups, however sesame oil (high in linoleic acid) was the only oil that showed a significant difference. (Agarwal, Gupta et al. 2000) In another randomized controlled trial, preterm and full term neonates were assigned to receive massage with coconut oil, mineral oil, or a placebo. Coconut oil massage resulted in significantly higher weight gain velocity compared to infants massaged with mineral oil and controls in the preterm infant group and greater weight gain velocity compared to controls in the full term infant group. The coconut oil group also had greater length gain velocity compared to controls in the preterm group. (Sankaranarayanan, Mondkar et al. 2005) A hospital-based study in Iran in neonates randomly assigned to massage with coconut oil, massage alone, and no massage, found a significant difference in weight gain between the oil massage and massage only group and between the oil massage and no massage group, but no difference between the massage only and no massage group. (Saeedi, Gholam et al. 2011) A study investigating the effects of cutaneous oil application on somatic growth and plasma linoleic and arachidonic acid levels in preterm newborns found the oil group had a greater increase in weight, length, arm circumference, and tricipital and subscapular skin folds compared to the group with no cutaneous treatment. However, an increase in linoleic acid and a decrease in arachidonic acid levels were seen in both groups. (Soriano, Martinez et al. 2000)

This improvement of nutritional status could be the result of transcutaneous absorption of essential fatty acids (EFA). Essential fatty acids are important components of a healthy diet as fats in the n-3 and n-6 families cannot be synthesized by the body. Linoleic acid (LA) and α-linolenic acid (ALA) are the parent fatty acids and must be obtained from food. Both are converted to longer chain more highly unsaturated fatty acids by enzymatic chain elongation and desaturation, which is required for barrier homeostasis. (Feingold 2007; Uauy and Dangour 2009) The pathway of EFA metabolism is shown in the Figure 2-2.

Sunflower seed oil is high in linoleic acid (about 65%) and low in oleic acid (about 20%). (Campbell 1983) Linoleic and arachidonic acids are essential long-chain polyunsaturated fatty acids, which may be deficient in infants. Deficiency can cause increased susceptibility to bacterial infections, failure to thrive, increased dermatitis, and thrombocytopenia. (Friedman, Shochat et al. 1976) Sunflower oil applications may vary the newborn's ability to mount a response to exposure due to a difference in systemic absorption of essential fatty acids, which may modulate immune function. (Kinsella and Lokesh 1990) EFAs are precursors for the synthesis of eicosanoids, which are polyunsaturated fatty acids that are precursors of prostaglandins and other enzymatic metabolites involved in the immune system. (Moncada and Vane 1978; Samuelsson, Dahlén et al. 1987) These mediators regulate many physiologic functions such as, inflammatory responses, thrombocyte aggregation, leukocyte migration, vasoconstriction and vasodilation, blood pressure, bronchial constriction, uterine contractibility, and apoptosis. (Uauy and Dangour 2009) Linoleic acid itself also has anti-inflammatory properties. (Schürer 2002)

If linoleic acid is deficient, oleic acid is converted to 5,8,11-eicosatrienoic acid by enzymes that usually produce arachidonic acid from linoleic acid. If linoleic acid deficiency occurs, it results in disordered lamellar bilayer structure and compromised epidermal barrier function. When oleic acid is substituted for linoleic acid, trienes derived from oleic acid may alter the fluidity and permeability of the cell. (Darmstadt 1998) The body compensates for essential fatty acid deficiency by converting fatty acids to their derivatives and accumulating mono-unsaturated fatty acids to maintain membrane fluidity. (Siguel, Chee et al. 1987)

Deficiencies in essential fatty acids can lead to changes in the skin and abnormalities in stratum corneum structure function. (Feingold 2007) Linoleic acid may be involved in the formation of the barrier layer of the stratum corneum, while arachidonic acid may prevent scaliness. (Prottey 1977) Fatty acids, particularly linoleic acid, increase the rate of barrier formation by activating peroxisome proliferator-activated receptor-α. (Darmstadt, Mao-Qiang et al. 2002) EFA deficiency can lead to dryness, desquamatization, and thickening of the skin and growth faltering. (Hansen, Wiese et al. 1963) EFA deficiency is characterized by reduced amounts of linoleic and arachidonic acid and excessive amounts of 5,8,11-eicostraienoic acid in plasma lipids. (Mead 1968; Sprecher 1972) A diagnosis of essential fatty acid deficiency is made using different clinical criteria such as a ratio of eicosatroenoic acid to arachidonic acid ≥ 0.4 (Darmstadt 1998), a ratio of Mead acid to arachidonic acid ≥ 0.025, or a ratio of total EFA to total non-EFA <0.6. (Siguel, Chee et al. 1987)

The two sources of linoleic acid are dietary fat and adipose tissue stores. Infants who are premature or small-for-gestational age have low body stores of essential nutrients and therefore are at greater risk for EFA deficiency. (Friedman, Shochat et al. 1976) The fat content of breastmilk is relatively constant at about 3-4% by weight with arachidonic acid being less variable than other fatty acids on a worldwide basis. (Brenna and Lapillonne 2009) During a child's first 6 months, dietary total fat intake should be between 40-60% of total caloric intake and 3-4.5% and 0.5% of total energy should be from linoleic acid and α-linolenic acid respectively. (Uauy and Dangour 2009) Most infant formulas add a mix of vegetable oils in order to have an adequate amount of EFAs. (Uauy and Dangour 2009) Preterm infants are the most susceptible to essential fatty acid and long chain polyunsaturated fatty acid deficiencies because of their limited fat stores and greater nutrient demands. Preterm infants supplemented with long chain polyunsaturated fatty acids (LCPUFAs) showed increases in growth. (Uauy and Dangour 2009) In fetal life, LCPUFAs, not the EFA precursors, are transferred across the placenta, so DHA steadily accumulates in the fetus during gestation. After birth, there is a rapid fall in circulating plasma LCPUFAs and a rise in linoleic acid. This may be adequate in full term infants however; preterm infants have minimal body fat stores in addition to dendritic and synaptic growth and development occurring. There is a high requirement of LCPUFAs and preterm newborn infants may not be able to elongate and desaturate EFAs at a fast enough rate. (Leaf 1996)

There is little knowledge of the prevalence of EFA deficiency in Nepal. A study completed on the nutritional status of rural Nepalese in southeast Nepal in the late 1980s found the mean polyunsaturated fatty acids (PUFA) and n-6 PUFA to be higher in males than in females, but both sexes had relatively low levels (30.4% and 31.6% of total fatty acids in females and males respectively). (Hirai, Takagi et al. 1996) When compared to the percentage of PUFA in patients with intestinal fat malabsorption (26%) (Siguel, Chee et al. 1987), it may indicate EFA deficiency in the Nepalese population. In addition, the ratio of EFA/non-EFA in this population was 0.42 for females and 0.45 for males, which is below Siguel's criteria of an EFA/non-EFA ratio >0.60 (Siguel, Chee et al. 1987), another indication of EFA deficiency in this population. (Hirai, Takagi et al. 1996)

Animal studies of EFA deficiency show abnormal epidermal permeability and barrier function and differentiation. These defects were corrected by both topical and systemic administration of linoleic acid or corn oil. Defects in barrier function were also reversed at the sites of the topical application of linoleic acid before the correction of the essential fatty acid deficient state showing that barrier function in EFA deficiency can be corrected locally without first showing systemic reversal. Topical application of olive oil (low linoleic acid) or oleic acid demonstrated deteriorated barrier function. (Elias, Brown et al. 1980) The cutaneous application of safflower oil to EFA deficient rats also corrected the plasma and erythrocyte biochemical abnormalities of EFA deficiency. The linoleic and arachidonic acid content of both the plasma and the erythrocyte phospholipid increased and the eicosatroenoic acid of both decreased. In addition, the linoleic acid content of plasma triglyceride increased with the safflower oil treatment. (Böhles, Bieber et al. 1976)

Case studies on the correction of EFA deficiency in newborns using cutaneous application of sunflower oil showed biochemical and clinical response within three to five days, with an increase in both arachidonate and linoleic acid and a decrease in 5,8,11-eicosatrienoic acid in plasma lipid levels. (Friedman, Shochat et al. 1976) Adults who acquired EFA deficiency after major intestinal resections had their deficiency corrected with only 2-3mg of linoleic acid per kilogram per day. (Press, Hartop et al. 1974) In patients with EFA deficiency, levels of epidermal lecithin increased, rates of transepidermal water loss decreased, and scaly lesions disappeared after 2 weeks of applying sunflower seed oil compared with no changes after application of olive oil. Control patients showed no changes after application with sunflower seed oil. (Prottey, Hartop et al. 1975) More recent studies have also found that the application of vegetable oils can improve serum fatty acid profiles. (Fernandez, Geetha et al. 2005; Solanki, Matnani et al. 2005)

Significance

Emollient therapy research to improve neonatal survival is a global priority. (Lawn, Zupan et al. 2006) Although millions of infants in South Asia are massaged with mustard oil each year, it is unknown whether substituting sunflower seed oil changes any biological mechanisms that may improve neonatal health. As massage of neonates with mustard seed oil is almost universally practiced in many communities in South Asia, neonates are routinely exposed to the effects of mustard oil massage. These effects could include increased risk of microfissures in the epidermal layer that might raise the likelihood of transcutaneous acquisition of invasive pathogens from the environment. (Darmstadt and Dinulos 2000; Darmstadt, Saha et al. 2003) Nutritional deficiencies could also cause compromised barrier function in these infants. Determining how and if certain biological mechanisms relating to skin barrier and integrity, microbial challenge, nutritional status, and innate immune responses are different in infants massaged with sunflower oil will help in the characterization of the modes through which sunflower oil massage may improve neonatal health, and allow for direct comparison of functional indicators across public health outcomes including neonatal skin infection, clinical signs of sepsis, and mortality. Such data are necessary to refine the timing, frequency, and mode of administration in future scaled up programmatic promotion of sunflower oil, a low cost (about $0.10 per massage) intervention with the potential to substantially improve survival of newborns across South Asia.

Chapter 2 Tables and Figures

Table 2-1: Potential Benefits of Topical Application of Emollients to the Newborn (Darmstadt and Dinulos 2000)

Potential Benefits of Emolliation Therapy	Possible Basis for Beneficial Effects
Reduced incidence of hypothermia	Ability to intercalate within stratum corneum to provide mechanical barrier to water loss
Improved nutrition through transcutaneous absorption of lipids	Affect lipid metabolism, highly active lipid metabolism in epidermis and the presence of a fatty acid transporter on keratinocytes make epidermis able to metabolize lipids from emollients (Feingold 1991; Schürer, Schliep et al. 1995)
Enhanced neurological development and promotion of mother-infant bonding through tactile stimulation	Activation of superior colliculus, responsible for organization of visual saccades (Vaivre-Douret, Oriot et al. 2009)
Normalization of TEWL	Ability to intercalate within stratum corneum to provide mechanical barrier to water loss
Improvements in skin condition (including hydration and surface lipid content)	Topical application of lipids directly provide metabolic building blocks to form healthy layers and repair damaged layers (Elias, Brown et al. 1980; Mao-Qiang, Elias et al. 1993)
Decrease of invasive infections in preterm infants (Nopper, Horii et al. 1996; Edwards, Connter et al. 2004; Darmstadt, Saha et al. 2005)	Fewer portals of entry for invasive pathogens

Table 2-2: Common Skin Flora Colonizing Newborn Skin (Sidbury and Darmstadt 2002)

Micrococcaceae
Coagulase-negative staphylococci
Staphylococcus epidermidis
Staphylococcus hominis
Staphylococcus saprophyticus (perineum)
Staphylococcus capitis (sebum-rich areas)
Staphylococcus auricularis (ear canal)
Peptococcus species
Micrococcus species
Diphtheroids
Corynebacterium (most intertriginous areas)
Brevibacterium (toe webs)
Propionibacterium (hair follicles, sebaceous glands)
Gram-negative rods
Acinetobacter (moist, intertriginous areas, perineum)
Rarely *Klebsiella, Enterobacter, Proteus*
Yeast
Malassezia species

Figure 2-1: Linkage Between Multiple Barrier Functions (Elias 2007)

A. <u>SINGLE STRESSOR CAN ALTER MULTIPLE FUNCTIONS</u>

1) ↑ Psychological stress → ↑ Endogenous steroids → ↓ Permeability barrier
↓ SC Integrity/Cohesion
↓ Antimicrobial barrier

2) ↑ pH → ↑ Serine protease activity → ↓ Permeability barrier
↓ SC Integrity/Cohesion
↑ Cytokine activation
↓ Antimicrobial barrier by allowing pathogen colonization and
↑ Degradation of antimicrobial peptides

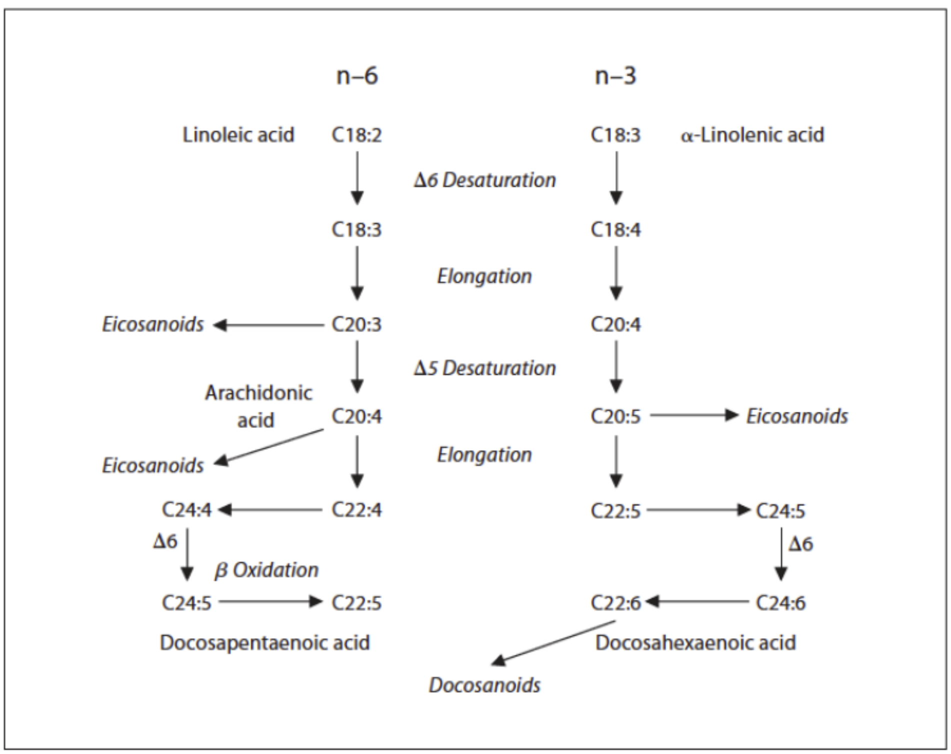
3) ↑ or ↓ SC hydration → ↑ or ↓ Permeability and antimicrobial barriers in parallel

B. <u>ALTERATIONS IN 1 FUNCTION CAN ALTER ANOTHER FUNCTION</u>

1) ↓ Mechanical strength → ↓ permeability barrier

2) Permeability barrier insults → ↑ antimicrobial and permeability barriers;
↓ resistance to percutaneous chemical/antigen/pathogen ingress
↑ inflammation

Figure 2-2: The Pathways to Convert LA to Arachidonic Acid (AA) and Docosapentaenoic Acid and ALA to Docosahexaenoic Acid (DHA) (Uauy and Dangour 2009)



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Chapter 3 Methods

This was a cluster-randomized community-based trial conducted by the Nepal Nutrition Intervention Project, Sarlahi (NNIPS) examining the biological mechanisms that may lead to improved health outcomes in neonates massaged with sunflower oil relative to mustard seed oil. It was nested within a larger parent trial on the Impact of Sunflower Seed Oil Massage on Neonatal Morbidity and Mortality in Nepal (NOMS), and hence the methods for the parent trial will also be discussed. NOMS was a cluster-randomized, community-based trial. Newborn infants were randomized within clusters (sectors) to receive either promotion of full body massage with sunflower seed oil or promotion of full body massage with mustard seed oil, which is considered standard in these communities and is almost universally practiced. The aims of the parent trial were to determine whether full body sunflower seed oil massage in newborns reduced neonatal morbidity or mortality in Sarlahi District, Nepal.

Study Population and Location

In 2011, the NNIPS surveillance area consisted of 26 Village Development Committees (VDCs) in Sarlahi District in rural Nepal. Each of these VDCs encompasses nine government defined geopolitical units (wards), which were further divided into sectors based on population. NNIPS has been conducting research in this area since 1989. Sarlahi is divided into a total of 96 VDCs and 2 municipalities, as defined by the government of Nepal, with NNIPS studies having been principally involved in the northern third of the district. The entire district has a population of over 600,000.

Sarlahi is a low, flat area in the Terai region of southern Nepal, lying along the border of Bihar State in Northern India. This study area's population of around 250,000 lives in rural farming communities comprised mainly of two ethnic groups, the *Madeshi* and the *Pahadi*. The *Madeshi* are from the southern plains and speak mainly Maithili and the *Pahadi* are from the hills and speak Nepali and other languages. Although the *Pahadi* are typically of a higher caste, the two groups have similar newborn care practices, with over 92% of births occurring in the home and more than 90% having no trained birth attendants at delivery. (Mullany, Darmstadt et al. 2006) This is considered a poor region of Nepal with a high rate of neonatal mortality (33 per 1000 live births) comprising more than two-thirds of all infant deaths. (Nepal Ministry of Health and Population 2011) The neonatal mortality rate measured in this area between 2002 and 2006 was approximately 38-40/1000 live births.

Procedures for Parent Trial

Sectors served as the clusters that were the unit of randomization for the NOMS study. The 9 wards in each of the 13 participating VDCs were divided into 1 or more sectors. On average, there were 1.6 sectors per ward and 14.4 sectors per VDC. Randomizing sectors rather than individuals helped to minimize crossovers or contamination of the intervention by providing each field worker only one type of oil to promote within her area. Previous sector level mortality estimates from prior research in this area were known for 9 of the 13 VDCs used in the NOMS trial and were used to perform restricted randomization in order to ensure balance of prior neonatal mortality risk. In the other 4 VDCs, sectors (clusters) were randomized with a computerized quasi-random number generator, stratified on VDC using blocks of 4, ensuring a geographical balance of the types of oil across the study area. Sector assignment lists were maintained at study headquarters in Kathmandu and Sarlahi. This clustered-randomization approach has been used in six major field trials in Sarlahi over the past 20 years. (West, Pokhrel et al.

1991; West, Katz et al. 1999; Christian, Khatry et al. 2003; Mullany, Darmstadt et al. 2006; Tielsch, Darmstadt et al. 2007; Tielsch, Khatry et al. 2007) A local resident woman, a “ward distributor” (WD), monitored each sector. All WDs worked within a defined sector (i.e. ward or subsection of a ward) and were responsible for identification, tracking, and following mothers and infants within their specific area. As such, any individual WD only promoted one type of oil and followed consistent promotion of the allocated oil throughout the duration of the study. Blinding was not possible given some differences in smell, texture, and taste between the two types of oils. While data collectors and higher-level supervisory staff were not directly aware of the sector-level allocations: it was not possible to fully mask the intervention in the NOMS study.

The nested mechanisms study took advantage of the randomization of the parent trial, using a subset of the VDCs in the original NOMS trial, which consisted of VDCs used in previous trials (sectors randomized using previous mortality estimates) and “new” VDCs (sectors randomized to achieve geographical balance). In the interests of efficiency and logistical constraints, VDCs were chosen that were closest to field headquarters, which facilitated efficient deployment of the biological mechanisms study field workers and more rapid return of the specimens to field headquarters, improving quality control of cold storage. Figure 3-1 shows the VDCs that participated in both the NOMS trial and the Biological (Oil) Mechanisms Sub-Study.

All households in the NOMS study area were mapped and entered into a population-based database. The total population in the proposed VDCs for the NOMS trial was approximately 100,000 with an annual birth cohort of about 3000 infants. All live born infants born in the study research area were eligible to participate in the NOMS trial.

Infants were enrolled if the caretaker provided consent for participation in the study and the local worker met the infant alive at the time of their first visit to the household.

For the NOMS trial, community-specific lists of married women of reproductive age were created. WDs carried out a combination of active and passive surveillance to identify pregnant women in the NOMS study area. Every five weeks, married women between 15 and 40 years were visited and queried about timing of last menstrual period; if no period had occurred since the prior visit, the woman was offered a pregnancy test. Other women who came into the area later in pregnancy to deliver (often at their maternal home, or *maiti*) were also eligible for enrollment. Regardless of mode of identification (i.e. active or passive), the WD explained the study and later returned with a “team leader interviewer” (TLI) to further explain the study and obtain consent. After a woman consented to be in the study, her pregnancy was tracked. Each consenting woman was given a set of common basic antenatal interventions including tetanus toxoid, a clean delivery kit, iron-folate supplements, chlorhexidine cleaning solution for disinfection of the umbilical cord, and basic educational messages.

Intervention

The worker visited the women in late pregnancy (~28-32 weeks) to promote the use of either the sunflower seed oil or mustard seed oil, and provide the mother or other caretaker with a 100ml bottle of oil at that time, along with guidelines for the use of the oil and massage technique. Formative research has indicated that about 10ml of oil is needed for each massage. After birth, larger quantities of oil (500ml in recyclable PET bottles and labeled with instructions) were provided at 3 intervals (usually day 1, day 10, and day 21 after birth), for a total of 1.5 liters during the neonatal period. A small finger bowl was also provided to each family. Each time the infant was massaged, a small amount of oil was transferred from the bottle to the finger bowl. Enough oil was provided for a full body massage of the infant 2-3 times per day for 28 days. Field workers visited homes every day during the first week of life to promote oil massage and address any questions or concerns of the mothers. Other possible caretakers including fathers and grandmothers were also given instructions on massage techniques.

High quality oil for a variety of uses including eating, cooking, and topical therapy was purchased from Shiv Shakti in Jitpur, Nepal. The company provided a manufacturing date imprint on the individually sealed packages and on large 20-liter containers and a recommended “use-by” date. Linoleic sunflower seed oil with high (about 65%) linoleic acid and low (about 20%) oleic acid was used. (Campbell 1983) The oil was purchased every 4 weeks and stored in sealed half-liter plastic packets at site headquarters at room temperature.

The company was required to submit a sample for purity/quality to the Government of Nepal Food Inspection Laboratory in Hetauda, Makwanpur District, Nepal. A copy of this analysis was received from the Hetauda food lab for each distinct batch. Determination of the fatty-acid composition of the oils was done at Geo-Chem Laboratories PVT LTD (Mulund, Mumbai, India). This fatty-acid profile analysis was repeated twice per year. These analyses consistently estimated the linoleic acid levels between 58%-67% and 18%-22% in the sunflower and mustard oils, respectively.

Data Collection

Household data were collected for all individuals during the first visit of the parent trial when mothers were enrolled, including socioeconomic status, education, household structure, maternal and paternal characteristics, and maternal health indicators such as reproductive history. After the birth, the caretaker initiated the full body massage using the provided oil following the guidelines provided by the WD. As soon as possible after birth or labor began, family members or neighbors notified the WD of the birth. The WD then visited the household for the next seven consecutive days to promote use of the provided oil and provide further guidance. At each of seven consecutive visits in the first week of life, the WD asked the number of times the infant was massaged since the previous visit and whether the oil provided by the project was used in order to measure compliance. A similar set of questions were asked by the TLI during their follow-up visits on days 1, 3, 7, 10, 14, 21, and 28. The TLIs also read and recorded the approximate volume of oil that was remaining in the bottle.

After conducting the first visit to the newborn, the WD recorded the location information, date and time of birth, and name of head of household on a form ("Birth Notification Form") and delivered this form to one of the drop boxes monitored by her supervising TLI. After receiving the Birth Notification Form, the TLI conducted an initial birth assessment visit. Using a standardized Infant Birth Assessment Form (IBAF) and Maternal Birth Assessment Form (MBAF) the TLI recorded information related to the mother and newborn such as late pregnancy morbidity, labor and delivery characteristics, birth assistance and practices, length of labor, maternal temperature, date and time of birth, sex, length, and weight of infant, and immediate newborn practice (thermal care, cord practices, early bathing and massage, breastfeeding initiation, etc.). Weight and length of the infant were also recorded.

After the initial birth assessment visit, the birth team member completed newborn follow-up (NFF) visits on days 1, 3, 7, 10, 14, 21, and 28. Figure 3-2 shows the overall study design and flow of women and newborns through the parent trial. At each visit, infant's vital status, respiratory rate, axillary temperature, signs of cord infection, chest indrawing, jaundice, and skin infection were recorded. Any maternal or caregiver reports of signs of morbidity including difficulty breathing, diarrhea, convulsions, perceived fever, etc. were also recorded. In addition, newborn care practices since the prior visit were collected, including breastfeeding and other feeding practices, topical application to the cord, bathing, and massage.

The underlying principles of scheduling these newborn follow-up visits were:

1. Get to the birth as soon as possible after it occurs
2. Maximize the number of visits where data were collected

NFF visits were scheduled as calendar date of birth plus day of visit (e.g. visit 7 would be scheduled on calendar date of birth plus 7). NFF visits were always recorded up until the date of the next scheduled visit. For example, the follow-up visit would be considered visit 7 if it were conducted any time between calendar date of birth plus 7 and calendar date of birth plus 9, after which it would be considered follow-up visit 10. If infants were not met on the day they were scheduled, workers would return to their homes in order to complete that visit, up until the day the next visit was scheduled after which the infant would be considered not met for the previous visit. Infants not met at the initial attempt of the 28-day visit were considered not met for their 28-day visit; workers did not return to their household. The scheduling of newborn follow-up visits for the biological mechanisms study followed these same guidelines.

If death of a newborn was confirmed during any of the follow-up visits, the deaths were reported to the supervisors immediately. The supervisor conducted a confirmatory visit to the household with the local WD after the mourning period in order to conduct a verbal autopsy interview with the parents or other caretakers. The verbal autopsy form used was based on the standard WHO instrument and included confirmation of date and approximate time of death, injuries, feeding and breathing patterns and difficulties prior to death, questions related to signs of tetanus, fever, diarrhea, dysentery, vomiting, cord and skin infections, and information on care-seeking patterns. Parents or other caretakers also provided an open description in their own words regarding the process of death for the infant. Verbal autopsies were reviewed independently by two Nepali physicians (co-investigators) familiar with the process. An immediate and one or more underlying causes of death were assigned. In the event of differences between the reviewers, a joint adjudication process resolved any remaining differences.

Procedures for Biological Mechanisms Sub-Study

This sub-study that is the focus of this dissertation included an extended set of measurements collected from a subset of infants participating in the main trial. The focus of data collection for this sub-study was on direct measurement of biological markers of infants built into a specific sub-study schedule (days 1, 3, 7, 14, and 28) of the parent trial visits, with an additional visit at 180-days. These biological measurements included: skin condition assessment, transepidermal water loss (TEWL), skin pH, and skin discs to evaluate skin integrity, skin swabs to examine bacterial colonization of the skin, salivary collection to assess immune status, serum collection to measure cytokines and fatty acids in the blood, and weight and length to measure nutritional status. Measurements were collected using tiered sample sizes in a way that the most invasive and time-consuming measures were done on the fewest number of infants (see Figure 3-3). For all infants enrolled in the mechanisms study the worker performed the following procedures: skin condition score (visits 1, 3, 7, 14, and 28), TEWL measurement (visits 1, 3, 7, 14, and 28), skin pH measurement (visits 1, 3, 7, 14, and 28), skin disc collection (visits 1, 7, 14, and 28), and salivary collection (visits 1, 7, and 14). In addition, in a subset of infants (n=400), skin swabs were collected (visits 1, 3, and 7) and an additional subset (n=200) had a heelprick done at visit 14. Weight and length were also collected by birth team members at visits for the parent trial (weight on days 1, 3, 7, 10, 14, 21, and 28 and length on days 1 and 28) and at the 180-day visit for the complete sample size. Table 3-1 shows the timing of visits for measurements taken during follow-up visits and the sample size for each measurement for the biological mechanisms study.

A subset of 7 VDCs of the 13 original VDCs in the NOMS trial was selected to participate in the mechanisms study, which began in July 2012. An 8th VDC was added after 5 months and a 9th VDC was added in January 2013 (see Table 3-2 and Figure 3-1). Among infants participating in the main study, determination of eligibility to additionally participate in the sub-study was done during the initial birth assessment visit, and was based on estimates of gestational age at birth. For this study, preterm infants (<37 weeks gestational age) were oversampled, aiming for approximately 50% of the sample to be preterm. During the study period (July 2012 until September 2013), the gestational age was estimated directly by the field worker using the date of last menstrual period estimated by the woman at the time of initial enrollment. Each birth team member had a list of the date of the mother's last menstrual period (LMP), which was used along with the date of birth to determine the gestational age of the infant. If the infant was born before week 37, one of the members of a specially trained team of field workers focused on the implementation of the mechanisms sub-study, was contacted directly by mobile phone. Infants born on or after week 37 were listed by VDC in consecutive order (i.e. by birth date) on a pre-printed computer generated blank form of 20 rows, 4 of which had been randomly selected for shading. Infants listed on a randomly shaded row were eligible for inclusion in the mechanisms study, thus selecting 4 out of every 20 (20%) of the full term infants for participation. All preterm (<37 weeks) infants and every 5th full term infant born in the mechanisms study area were eligible for enrollment in the sub-study.

After the birth team member determined a newborn was eligible for inclusion in the biological mechanisms study, the mechanisms worker visited the home and initiated a process to obtain informed consent for the newborn’s participation. Eligible infants were enrolled if the caretaker provided consent for participation, the birth team member met the infant alive, and the mechanisms team member was able to enroll the infant within the first 48 hours after birth.

On the initial visit to the home by the biological mechanisms field worker, a consent form was read before collecting any information. The consent statement described the mechanisms study’s purpose and procedures that were carried out at each of the visits. It described that, in addition to the procedures for the main study, some additional measurements would be carried out on the infant that would help to explain more about the differences between mustard oil and sunflower oil. There were additional explanations read if the visit was to include skin flora collection and/or blood. It also explained that there were no significant risks to being in the study, and that all information would be kept confidential. Voluntariness and who to contact was also explained. Each field worker had a laminated copy of the approved oral consent script in Nepali, and the process was documented on standardized data collection forms. The Nepal Health Research Council and the Committee on Human Research of the Johns Hopkins Bloomberg School of Public Health approved this study.

Once consent was granted, the mechanisms field worker continued with follow-up assessments scheduled for days 1, 3, 7, 14, and 28. Depending on the day of the follow-up visit, different measurements were taken as described above (see Table 3-1). At these visits the team member also asked additional interview questions about oil massage, feeding, and bathing practices.

The sequence of measurements were as follows:

1. Interview questions
2. Skin Condition Assessment
3. Skin Swab Collection (if scheduled)
4. TEWL
5. Skin pH
6. Skin Disc Collection (if scheduled)
7. Salivary Collection (if scheduled)
8. Blood Collection (if scheduled)

All data were collected on standardized data collection forms.

Birth team members collected the additional measurements of weight and length at follow-up visits for the parent trial. During the initial visit, this information was recorded on the IBAF and the birth team member initiated a new form for recording the weight and length for the subsequent visits (see Table 3-1). In order to schedule the 180-day follow-up visit, a list by VDC of all infants who should receive a 180-day follow-up visit during each week was generated at the data center in Kathmandu. These lists were generated for a full Nepali month on the 15th of the preceding Nepali month and provided to the birth team members. At the beginning of each week, the birth team members identified which infants were scheduled to receive their 180-day follow-up visit. If no one was met within four weeks of the scheduled visit week, the infant was considered not met for his or her 180-day visit. Vital status for the mother and infant were also recorded at this visit.

Measurement Methods

Specific Aim 1: Skin Integrity and Barrier Function

Four measurements were used to assess the relationship between the type of oil used for newborn massage and skin barrier function and integrity. These were: skin condition score, TEWL, skin pH, and stratum corneum protein content. Prior to the start of the study, measurement techniques for TEWL, skin pH, and skin disc collection were piloted on adult forearms in order to determine if skin should be wiped with any materials prior to measurements being taken. Both mustard oil and sunflower oil were tested on different arms measuring TEWL, skin pH and protein concentration after oil was applied without wiping and after wiping with gauze, alcohol swabs, and cotton at 4 different locations on the arm. A decision was made based on the data not to wipe the skin as it might affect the skin barrier prior to measurement and to examine the effect of time since massage during analyses.

1. Skin Condition Score:

Field worker evaluation of skin condition was measured using a modified version of a scoring method described by Visscher et al. (Visscher, Odio et al. 2009) This team has experience extending these scores for patients in the United States. This study used an extended version of this more established scale for the measures of dryness, erythema, and rash (see Table 3-3), rating erythema severity on a scale from 0 to 3 and area affected on a scale from 0 to 4. Severity of rash and area affected by rash were evaluated on scales from 0 to 4 and 0 to 6 respectively. Dryness severity was evaluated on a scale from 0 to 5 and dryness area was evaluated on a scale from 0 to 3.

The first step of training in the use of the scales involved the field workers evaluating four sets of photos of infants in the population with varying degrees of skin condition. These photos were first evaluated and given a score for each skin condition component by an expert on skin condition evaluation. Each worker's scores were then compared to this master key. After 4 days of training, percent agreement for each worker compared with the key was above 70%. Unfortunately this way of training was somewhat limited as the workers were viewing the photos on computers, with some photos being of poor quality and with non-ideal lighting conditions, making it sometimes difficult to judge the color of the skin. Additional training was then completed in the field with older infants prior to the start of the study. During the study, this author also evaluated skin condition at each supervised field visit. The percent agreement between field workers and this author during the study period was close to 95%.

This assessment occurred on days 1, 3, 7, 14, and 28. Three areas of the body were chosen as areas to represent the infants' skin condition. These areas were the mid-chest between the nipples, the entire left arm, and the entire right leg. The area inside a 4cm by 4cm template was evaluated for the chest area. The chest area was evaluated first, followed by the arm, and then the leg. If the infant was crying or distressed, the workers waited until the infant was calm to complete the assessment. The infants' skin assessment scores for severity and area for each component (erythema, rash, and dryness) were recorded separately on standardized data collection forms.

2. TEWL:

TEWL was measured at visits on days 1, 3, 7, 14, and 28. Measurements were taken three times at the mid-chest nipple line. The exact location where measurements were taken was similar in all infants, through the measurement of anatomical markers. The location of the measurements was chosen, because it was easily accessible, an area that was massaged, and provided a reasonably flat surface area for placing the instrument. Measurement of transepidermal water loss was measured in $g/m^2/hr$ using a VapoMeter (Delfin Technologies, Ltd, Finland), a closed-chamber device containing sensors for relative humidity and temperature, using standardized procedures. (Rogeries and Group 2001; Nuutinen, Alanen et al. 2003; De Paepe, Houben et al. 2005) Once the VapoMeter contacts the skin, the relative humidity in the chamber begins increasing. The TEWL value is calculated from the information the instrument acquires based on this increase. Before each measurement the workers wiped the area of the VapoMeter that came in contact with the infant's skin with an alcohol swab. The three measurements along with the temperature and relative humidity obtained from the VapoMeter were recorded on standardized data collection forms.

3. Skin pH:

Measurements of skin pH were performed with a flat electrode (Skincheck™, Hanna Instruments, UK) calibrated daily to pH 4 and 7. (Parra and Paye 2003) These calibrations were done at field headquarters and were recorded each morning before the worker left to complete their house visits. Measurements were taken in the same location at the mid-nipple as for the TEWL measurements. Before each measurement was taken, the worker cleaned the electrode with an alcohol swab and rinsed it with distilled water before placing on the chest. Three measurements were taken and recorded on standardized data collection forms.

4. Stratum Corneum Protein Concentration:

Using a technique described by Voeglei et al., protein content from the outermost stratum corneum was analyzed using samples collected with 380 mm^2 D-Squame adhesive discs (CuDerm, TX) applied to the mid-chest with constant pressure for 2 minutes. (Voegeli, Heiland et al. 2007) Different locations on the chest were used for the placement of the skin discs at each of the four visits (days 1, 7, 14, and 28) in order to collect discs from skin that had not been previously exposed to adhesive. These sites were different from those used for TEWL and skin pH measurements. Before handling the discs the workers put on a pair of powder free gloves. They then used tweezers to remove the disc from its backing and place it on the chest where they applied gentle pressure by rubbing the disc clockwise three times with their thumb. After two minutes, the worker used the tweezers to remove the disc from the chest and placed it in a 2ml microtube with the adhesive facing inward. These microtubes were labeled with a unique 6-digit identification label, with an identical label placed on the data collection form. The microtubes were then transferred to field headquarters in cold boxes kept between 2° and 8°C. At field headquarters, the workers again put on powder free gloves and removed the skin discs from the microtubes using tweezers, placing them on a plate to determine the optical density. The optical density of the skin discs was determined using a spectrophotometer SquameScan™ 850A (Heiland electronic, Wetzlar, Germany) specifically designed for the application of D-Squame discs. Before the optical densities of the discs were read, the spectrophotometer was calibrated to 0% and 36.2% optical densities (a daily calibration record was maintained throughout the entire study). Optical densities of the discs were recorded on standardized data collection forms. After they were recorded the discs were placed back in their microtubes and stored in liquid nitrogen for further processing relating to the measurement of epidermal cytokines (see below). The following equation was used for quantification of protein concentration: (Voegeli, Rawlings et al. 2007)

$$C_{protein}\left(\frac{\mu g}{cm^2}\right) = 1.366 * Absorption(\%) - 1.557$$

Specific Aim 2: Nutritional Status

There were three measurements used to assess the relationship between the types of oil used for newborn massage and a neonate's nutritional status: weight at days 1, 3, 7, 10, 14, 21, 28, and 180, length at days 1, 28, and 180, and serum fatty acid levels collected at day 14. Questions regarding massage practices between months 1 and 6 were also asked during the additional follow-up visit specific to the mechanisms study after 180 days. Although in these analyses only weight and length up to day 28 were analyzed, methods for serum fatty acid collection will also be discussed.

5. Weight and Length

The birth team member for the NOMS trial performed weight measurements at the homes during field visits at the initial birth assessment (day 1) and at follow-up visits on days 3, 7, 10, 21, 28 and 180 and length measurements at the birth assessment (day 1) and at follow-up visits 28 and 180. Weight measurements were taken without clothes using a Tanita portable digital infant scale (Tanita BD-585, Tokyo, Japan), with accuracy to ±10g. Length measurements were taken using a locally manufactured wooden length board.

6. Fatty Acid Levels

Field workers collected samples of serum in order to assess fatty acid levels on day 14 via heelprick using BD Quikheel lancets (BD Quikheel™ Lancets, Becton, Dickenson, and Company, Franklin Lakes, New Jersey). After putting on gloves, the workers rubbed the right heel gently for 30 seconds in order to warm the area and encourage blood flow. They then wiped the medial part of the right heel with alcohol allowing the alcohol to dry. The lancet was positioned against the medial part of the infant's right heel (the area that was disinfected) at a 90-degree angle to the length of the foot. After pressing the trigger of the lancet firmly, pressure was applied so drops of blood would form, which were collected in a microtube placed at a 30- to 45-degree angle to the foot. If a complete microtube was filled, a second microtube was placed to collect the blood. After the blood was collected, the area was cleaned with sterile gauze, the foot was slightly elevated, and a small bandage was placed over the incision. A unique 6-digit identification label was placed on each microtube, with a matching label placed on the standardized data collection form. The microtube(s) were inverted 10 times to disperse EDTA, an anticoagulant coated along the sides of the microtubes. The tube(s) were then placed in a cool box at 2° to 8°C for transport to field headquarters.

The red blood cell pellet was separated from the plasma at field headquarters by trained laboratory technicians. Upon reaching field headquarters, each microtube of blood was centrifuged for 10 minutes at 1677 x g (5000 rpm). After centrifugation, the plasma was removed with a small plastic transfer pipet to a 1.5ml cryovial. This cryovial was then given a unique 6-digit label, with its duplicate being placed on the data collection form. Using the same plastic transfer pipet, the buffy coat was removed and discarded into a 10% bleach solution. If the sample of blood was too small, the removal of the buffy coat was skipped. The red blood cells were then washed by adding 0.5ml of cold 0.01M phosphate buffered saline, pH 7.4 with a clean micropipette tip and inverting the tube 4 or 5 times to gently mix the solution with the red blood cells. The tubes were then centrifuged again for 10 minutes at 1677 x g (5000 rpm). Using a fresh plastic transfer pipet for each sample, the saline and remaining buffy coat was removed and discarded into the 10% bleach solution. The viscous washed red blood cell pellet was then transferred to a second 1.5ml cryovial. An identical label as was placed on the microtube in the field was then placed on the cryovial. Each sample yielded two cryovials, one containing plasma to be used in examining serum cytokine levels (discussed below) and one containing the red blood cells, which was used to examine serum fatty acid levels. Both samples were stored at -15° to -20°C until shipped to collaborating laboratories at the Kennedy Krieger Institute (for fatty acids) and Johns Hopkins Hospital (for cytokines) in Baltimore, Maryland for analysis.

Specific Aim 3: Microbial Challenge

One measurement was used to assess the relationship between the type of oil used for newborn massage and microbial challenge, skin swab collection to examine the density and types of bacterial colonization on the skin. These data were not analyzed as a part of this dissertation, however the methods for collection will be discussed.

7. Skin Colonization

Specimens of resident cutaneous flora were collected from the axilla, abdominal, and inguinal areas using surface swabs on visits 1, 3, and 7. Specimens were collected from a 4cm x 4cm area of skin, with the area to be swabbed indicated with a locally made, disposable template. The worker first collected skin swabs from the right axilla area. The templates were sanitized with alcohol swabs and placed on the area to be swabbed.

After putting on gloves, the worker unwrapped a sterile swab from its package and dipped it into a tube filled with a phosphate-buffered saline (PBS) solution. Both the PBS solution and the STGG transport media (see below) were transferred from field headquarters to the house visits in cold boxes kept between 2° and 8°C. The swab was pressed against the wall of the tube to squeeze out any extra solution after which the worker rubbed the swab 5 times horizontally and 5 times vertically over the area within the template. Holding the swab with one hand, the other hand was used to open a specimen tube containing liquid transport media (skim milk, tryptone soya, glucose, glycerol [STGG]. (Gibson and Khoury 1986; O'Brien, Bronsdon et al. 2001) The swab was immersed in the media in the tube and the tube was placed in the same cold boxes kept between 2° and 8°C for transport back to field headquarters. Each tube was labeled with a unique 6-digit label number, with an identical label placed on the standardized data collection form. The same technique was repeated for the right inguinal and supra-umbilical areas. Samples were stored in liquid nitrogen at -20° to -80°C at field headquarters and were transported on a bi-weekly basis to our collaborating microbiology laboratory at the Institute of Medicine, Tribhuvan University in Kathmandu, which also prepared the PBS solution and STGG transport media that were sent bi-weekly from Kathmandu to field headquarters.

Specimens were transported to Kathmandu in liquid nitrogen for processing and stored in liquid nitrogen at the laboratory in Kathmandu. When it was time for processing, specimens were removed from cold storage and the swabs were vortexed in a PBS buffer solution. Sheep blood and MacConkey agar plates were inoculated with 10µL of the neat specimen and three consecutive 10-fold dilutions and incubated overnight at 37°C. Complete colony counts and bacterial identifications of sensitivity of isolates were done within 24 hours by standard biochemical and staining procedures. Colony count was done as per the following formula:

$$\frac{Bacteria}{cm^2} = \text{No. of colonies in } 10\mu\text{L of PBS} * \text{dilutions} * 6.25$$

Colony counts and types of bacteria identified were recorded on standardized data collection forms.

Specific Aim 4: Systemic and Epidermal Innate Immunity

There were three measurements used to assess the relationship between the type of oil used for newborn massage and systemic and epidermal innate immunity in the neonate: levels of serum and salivary cytokines and levels of structural and immune biomarkers of the skin (including cytokines). The field workers collected salivary samples on days 1, 7, and 14, skin discs on days 1, 7, 14, and 28, and performed heelpricks to collect serum on day 14. These data were not analyzed as a part of this dissertation, however the methods for collection will be discussed.

8. Serum Cytokines

Field workers collected samples of blood at homes in order to assess serum cytokines on day 14 via heelprick. These methods were discussed in the section on the collection of serum fatty acid levels above. After shipment to our collaborating laboratory at Johns Hopkins Hospital in Baltimore, Maryland, samples will be analyzed for the serum cytokines to be measured, which include IL-1α, IL-1β, IL-6, IL-8, TNF-α, by immunoassay.

9. Salivary Cytokines

Field workers collected samples of saliva at homes in order to assess salivary cytokines on days 1, 7, and 14 using Salimetrics Infant's Swabs (Salimetrics LLC, State College, PA), which can usually recover a volume of saliva in the range of 200-1000μL. Tests using a similar microsponge have shown that the time to acquire a minimum sample test volume for a typical salivary assay of 25-50μL was 20-30 seconds. (Granger, Kivlighan et al. 2007) Salivary collection was done at least 20 minutes after the infant was last fed to avoid any contamination of cytokines present in the mouth from breastmilk. Before salivary collection, the workers put on gloves and removed a new swab from its packaging. The swab was folded in half and placed in the infant's mouth, under the tongue, so both ends of the swab were inside the mouth and the worker was holding the folded middle portion. Swabs were left inside the mouth for 2 minutes in order to collect an adequate amount of saliva. After 2 minutes, the worker removed the swab and cut it in half, cutting off the middle portion that was dry where the worker was holding it. The worker then removed the needle of a 3cc syringe and placed the first half of the swab into the syringe. The syringe was then depressed, squeezing the saliva from the swab into a 2ml microtube. The swab with the saliva extracted was then removed from the syringe and placed in a second microtube. This method of saliva extraction was repeated for the second half of the swab. If less then 50μL (about 4 drops) of saliva was extracted, a second salivary sample was collected using the same process. Each microtube was labeled with a unique 6-digit identification number with an identical label placed on the data collection form. Immediately after collection, samples were placed in a cool box kept at 2° to 8°C for transport to field headquarters. The samples were then stored in liquid nitrogen at -20° to -80°C. Samples were shipped to the Center for Interdisciplinary Salivary Bioscience Research in Tempe, Arizona for analysis and will be analyzed for the salivary cytokines to be measured, which include IL-1α, IL-1β, IL-6, IL-8, TNF-α, by immunoassay.

10. Structural and Immune Biomarkers:

Structural and immune biomarkers were measured from the outermost stratum corneum using the same noninvasive sampling method as discussed above for the collection of skin discs for measurement of stratum corneum protein concentration (via optical density) and described by Narendran et al. (Narendran, Visscher et al. 2010) The same skin discs were used that were collected for the stratum corneum protein concentration collection using 380 mm^2 D-Squame tapes (CuDerm, TX) applied at the mid-nipple line with constant pressure. These clear polymer discs uniformly sample a fixed area of the skin surface, removing the stratum corneum in a reproducible way for analysis. After the field workers measured the optical density, the discs (placed in the microtubes) were stored in liquid nitrogen and shipped to a collaborating laboratory for further processing.

Handling, Storage, and Transport of Specimens

In order to preserve the integrity of the specimens that were collected during this study, while in the field specimens were kept in cold boxes at less than 8°C. LogTag temperature monitoring devices (LogTag TRID30-7, LogTag Recorders Limited, Auckland, New Zealand) were placed in each of the cold boxes to monitor the temperature throughout the day. Each morning the field worker recorded the date, time LogTag device was turned on and placed in cold box, device ID, and cold box ID. After returning from the field, the time the LogTag devices were removed from the cold box and turned off was recorded. The devices checked the temperature of the cold boxes at 2-minute intervals, storing the data, which was downloaded at the end of each workday to a central computer at field headquarters.

Once specimens were brought to field headquarters and processed, each specimen was recorded in a log specific for specimen type (skin swabs, skin discs, plasma, saliva, salivary swabs, and red blood cells). This log contained the label number of the specimen, specimen type, collection date, the bag number the specimen was stored in the liquid nitrogen tank, and also the route and shipment date (these were filled when the shipment was sent to Kathmandu). Each specimen storage bag only contained one type of specimen and contained a maximum of 45 specimen tubes. Cellophane was wrapped around each label before the tubes were placed in the liquid nitrogen to ensure the label would not detach. When the shipper tanks were ready to be sent to Kathmandu, the route and shipment date were recorded on the logs and transmittal lists of all specimens being sent were generated. These lists were checked for any potential problems (duplicate label numbers in current and previous shipments) and afterwards specimens were shipped to Kathmandu and then to their respective laboratories.

Forms Management

A NOMS Data Folder was initiated for every enrolled pregnant woman upon completion of her Late Pregnancy Visit (LPV). The outside of the folder had the identification information of the enrolled woman, her husband, her household, and her place of delivery. The outside also tracked the status and number of all the activities/forms completed for enrolled women and their infants from LPV to the end of study participation. All folders were held securely in the VDC office where the enrolled woman and her infant(s) resided. At the end of study participation the outside of the data folder was completed to indicate which forms were not completed and the number of forms within.

Forms from the mechanisms study were also added to these folders, however, during data collection for the neonatal period, the forms for the mechanisms study were kept at field headquarters. If an infant was enrolled in the mechanisms study, the birth team member placed a red sticker in the upper right hand corner of that NOMS folder. At the end of each day, the mechanisms workers brought the forms that were completed in the field back to field headquarters. Infants who were newly enrolled were recorded in logs for numbers of preterm and full term infants, depending on their gestational age. These forms kept a running list of how many infants were enrolled in the study to monitor when the 500th infant was reached for each group. It also allowed monitoring of the number of infants who had received heelpricks and skin swabs, since these were only done in a subset of the study population.

On the day of the first visit, each infant had a folder initiated at field headquarters with the identification information of the enrolled woman, her household, and her child included. The status and number of all the activities/forms completed for the mechanisms study were also tracked on this folder. When the NOMS data folder arrived from the VDC office with a red sticker in the right hand corner, the forms from the mechanisms study folder were transferred to the NOMS data folder. The NOMS data folder was then sent to NNIPS Headquarters in Kathmandu for data entry. Completed forms recording information for the 180-day follow-up visit were kept in separate folders in each VDC office. Each week they were transferred to the NNIPS field headquarters and checked for completion. They were then sent to NNIPS Headquarters in Kathmandu for data entry.

Oil Storage and Packing

In order to preserve the integrity of the oil until the participating households received it, all study oil, until day of distribution to the field, was stored and bottled in an air-conditioned, temperature-monitored room. The optimal temperature range for oil storage is between 20-28°C (Rajendra Acharya, Hetauda Government Food Laboratory Chief, personal communication). Therefore, the maximum and minimum temperature in the oil storage and packaging room over a 24-hour period in the room was recorded daily by oil handling personnel. In addition, the study oil bottles, 500ml and 100ml, were filled on two, physically separated flat surfaces. Each oil type had its own necessary packaging equipment that was thoroughly cleaned between oil batch purchases.

The labels affixed to each bottle were sticky on one-side and pre-printed on water-resistant paper. Printed on the label were the NOMS logo of a mother massaging her infant, the NNIPS logo, and the NNIPS organization name written in Nepali. The 500ml bottles used in the study were of the same size and all parts were made of plastic (bottle, inner lid and cover). The Batch Number was also written on the labels in waterproof marker according to the type of oil that would be poured into the bottle and the current NNIPS week (e.g. mustard seed oil and NNIPS week 054: “M054”) left to right with M/S placed at the top of the label. Before the bottle was filled, the cover and inner lid of the bottle were removed and the bottle was examined to ensure the inside of the bottle was clean and dry. The bottle was placed on the packaging table with a funnel placed in the opening. One 500ml factory packet was used per bottle, with the worker checking to make sure the oil in the packet matched the oil type of the Batch Number written on the label. After the bottle was filled, two circles of thin plastic were placed on the top of the bottle, with the inner lid placed on top, and the cover was screwed on tightly. The number of 500ml bottles filled each day were recorded in a daily log book. Similar procedures were used to fill the 100ml bottles, however the oil from the 500ml factory packet was poured into a small, plastic pitcher and the bottles were filled from the pitcher.

Oil Tracking and Distribution

In order ensure the oldest batches of oil were bottled and distributed to households first and no excess oil was removed from the temperature-controlled environment, a number of methods were employed. When a new oil purchase arrived at field headquarters, it was immediately placed in the oil storing and packaging room. At that time, the details of the purchase were recorded. Each week, oil was packaged and sent to the field to fulfill the study's oil needs the following week. All of the oil from one oil purchase was packaged before a new purchase arrived at the main study office so that no Bottle Code was assigned to bottled oil from two different purchases. Packaged oil was sent, the oldest packaged bottles first according to their Bottle Code, from field headquarters to the VDC offices on Wednesday of every week. The VDC's oil supply was distributed to households using the oldest packaged bottles first. Pregnant women received 100ml of oil at the late pregnancy visit. One 500ml oil bottle was provided per live birth on the first day of life, one on Day 10 NFF visit, and one on Day 21 NFF visit. If at any NFF visit the oil level in the 500ml bottle was found below three centimeters, the TLI brought a new 500ml bottle of oil on the next NFF visit. This distribution design ensured the households a constant supply of oil.

Quality Control

The NNIPS study team used a supervisory structure that helped to ensure that quality data was collected in the field. A structural hierarchy was in place with checks and balances to ensure data quality. WDs who monitor the pregnancies and act as the primary contact between the mothers and newborns and the study staff, conducted the first level of data collection. All of these women were literate and had more than 10 years of experience working on community trials. WDs identified and maintained a list of pregnant women in each sector, provided the massage oil and oil promotion, and accompanied the team leaders (TLIs) and birth team members on follow-up visits.

The team leaders supervised 8 to 14 WDs. These TLIs were men and women who had worked with the NNIPS team on previous studies and who showed good leadership and organizational skills. TLIs were directly responsible for enrollment, birth assessment, and the newborn follow-up visits at days 1, 3, 7, 10, 14, 28, and 180. They provided oversight in preparing the WDs schedules and distributed and maintained data collection forms at the VDC level. They reviewed all forms for missing or inconsistent data and revisited a subset of households to ensure the consistency of the WDs data. Every Thursday, they held a weekly meeting with all of their WDs to identify any problems in the field and to discuss field activities.

Field supervisors, all of who had more than 15 years of experience with the organization and other large-scale health programs in rural Nepal, provided the highest level of supervision. They were in charge of managing any major problems that arose as well as provided support and oversight to area coordinators. They were also responsible for assisting with the design, pretesting, and refinement of the data collection instruments and procedures and for organizing and conducting training programs for all field workers. In addition, this author assisted in supervisory visits to the field at least one day per week throughout the time of the mechanisms study period in which data for this dissertation is presented, observing each of the five biological mechanisms field workers at least once per month and ensuring standardized measurement procedures and data collection.

Once data were received at the central data processing unit, the forms were again checked for completeness and data entry operators entered the forms into the database on a series of networked client personal computers. Data entry screens displayed identification information on the screen to ensure that information was being entered for the correct individual. Data that were critically important were entered twice and confirmation was necessary before the database could be updated. Automated range and consistency checks were programmed into the database in order to ensure that the correct forms were linked to each participant. Data entry validation procedures were conducted at regular intervals to track the rate of data entry errors in order to identify the need for retraining. If problems were identified during data entry or data cleaning, those that could not be resolved by reviewing the form were returned to field supervisors who communicated with the workers in the field. The corrected forms were then returned to the central data processing unit for editing. All problems identified were documented through an electronic audit trail. Those ongoing checks helped to improve data quality by keeping the database up to date and by allowing the identification of reoccurring problems that indicated a need to revise the data collection forms or procedures.

All data were backed up at the end of each workday on external storage devices and a backup server was used to protect against server failure. Both the primary and backup servers had mirrored drives to protect against drive failure. All servers were located in field and project headquarters, which were locked and guarded at all times. Encrypted study databases were transferred to Baltimore on a regular basis and those files were kept on our server behind a local firewall. All accounts and database files were password protected.

Sample Size and Power Calculations

This nested biological mechanisms study included a subset of the sample size of 29,620 infants required for the parent trial. Some measurements that were collected were fairly easy to obtain as they were noninvasive measures or procedures and as such did not impose a great burden on the mother or the infant (i.e. weight and length measurements). However, some measures were much more time consuming and invasive. Also, some measures were more exploratory and therefore it may not have been necessary or ethical to perform those measurements on a large number of infants (i.e. fatty acid levels, and serum and epidermal cytokines). Therefore, we tiered the sample sizes in a way that the most invasive and time-consuming measures were done on the fewest number of infants (see Figure 3-3). As there was little to no information available on these measurements in newborns, any information gathered will make important contributions, as it will help direct further research in determining whether larger studies should be conducted. It will, however, be informative to have all of the measurements performed on the same set of infants, in order to determine how and if any of the biological mechanisms are related, as such, there were 200 infants who had all measurements taken (see Figure 3-3).

We hypothesized that the outcome measures (and the moderating effect(s) of the different oils, if any) may be more important in preterm infants than full term infants due to the immaturity of their skin, therefore, preterm infants were oversampled. A total sample size of 1000 infants was selected for Tier 1 measurements (see Figure 3-3), equally stratified by 500 preterm and 500 full term infants. In doing so, the sample of this study did not reflect the actual preterm/full-term ratio of the study population.

All power calculations assumed a design effect of 1.5, a 5% loss to follow-up, and a 5% Type 1 error. A design effect of 1.5 was assumed without prior knowledge of the true extent of correlation of the different measurements within clusters. The analyses presented here represent a preliminary analysis of the first 63.7% of the anticipated enrolled infants.

Sample Size as Related to Specific Aims

Specific Aim 1: Skin Integrity and Barrier Function

All measurements related to skin barrier integrity and function were located in Tier 1 with 500 infants in each group (see Figure 3-3). Comparing differences in mean TEWL, skin pH, protein concentration, or skin condition scores between groups at each visit we should have been able to detect even a small difference in means with high power, however as can be seen in Table 3-4, as the standard deviation of the mean increases, the power to detect a difference decreases.

At birth, the skin surface is relatively neutral (pH about 6.5) and gradually becomes more acidic over the first few postnatal weeks. The acid mantle forms as a result of changes on the skin surface following birth (sweat, sebum, microorganisms) and lactic acid and free fatty acids from metabolic processes within the stratum corneum. The skin pH falls to about 5.7, a level that is beneficial for antimicrobial defense by inhibiting the growth of pathogenic bacteria. (Yosipovitch, Maayan-Metzger et al. 2000; Hoeger and Enzmann 2002) For this measurement we were interested in determining whether there was a difference in the time it took for a newborn's skin to reach a beneficial pH level of 5.7. Assuming a hazard ratio of 0.8 and an equal proportion of infants in both groups, we had 80% power to detect a difference in the survival curves.

Specific Aim 2: Nutritional Status

There were three measurements related to determining differences in the nutritional status between the two groups. Two of these measurements were Tier 1 measurements and one was a Tier 3 measurement (see Figure 3-3).

The two measurements related to nutritional status that were Tier 1 sample size measurements were weight and length. For these measurements we were interested in comparing the proportion of infants who were less than 2 standard deviations below the normal mean weight-for-age (underweight) for each measurement point (girls: 3.2 kg, boys 3.4 kg at 28 days and girls: 5.7 kg, boys 6.4 kg at 180 days) and height-for-age (stunted) (girls: 49.8 cm, boys: 50.8 cm at 28 days and girls: 61.2 cm, boys 63.3 cm at 180 days) according to the WHO (see Table 3-5). (WHO 2011) We were also interested in looking at the differences in the mean weight and length measures for each visit comparing oil groups, which we would have the same power to detect with 1000 infants as we had for the skin integrity measures shown in Table 3-4.

The third measurement related to nutritional status was the measurement of fatty acid levels of the infants, which due to the invasive nature of the measurement and that it was more exploratory, was a Tier 3 measurement. The measurement of interest was whether there was a difference in the mean percentage of total fatty acids that was linoleic acid. (Siguel, Chee et al. 1987) With a difference in mean percentage of fatty acid of 5% we would have 81-100% power to detect a difference with this sample size if the standard deviation was between 5 and 10% (see Table 3-6).

Specific Aim 3: Microbial Challenge

The measurement of skin flora type and amount is related to microbial challenge and was a Tier 2 measurement (see Figure 3-3). The measurement of interest was a difference in the mean amount of bacterium present on the skin when comparing infants massaged with mustard seed oil with those massaged with sunflower oil. The differences in means were based on a study done by Pabst et al., comparing bacterial colonization of the skin of infants massaged with Aquaphor with a control group. This study was done in very low birthweight infants, so the differences observed may be greater. (Pabst, Starr et al. 1999) However, even with a very low difference of 0.2 $\log_{10}$ CFU/ml, if the standard deviation was low, there was high power to observe a difference (see Table 3-7).

Specific Aim 4: Systemic and Epidermal Innate Immunity

There were three measurements that related to systemic and epidermal innate immunity. One of these measurements was a Tier 1 measure and the other two were Tier 3 measurements (see Figure 3-3).

The collection of salivary samples was a Tier 1 measure, however, a full analysis of the cytokines was only completed on a subset of 400 out of these 1000 infants. Of interest was the determination of a difference in the mean amount of certain salivary cytokines. As there was no information on amounts of cytokines measured in saliva for infants we used as our proxy to estimate the differences in means and standard deviations a paper by Skogstrand et al. which measured cytokines present in serum in preterm and normal term infants (see Table 3-8). (Skogstrand, Hougaard et al. 2008)

Serum cytokine levels were a Tier 3 measurement. The measures of interest were the types and amounts of different cytokines and whether they were different between groups. In estimating the differences of the means and the standard deviations we again used information from the Skogstrand study. (Skogstrand, Hougaard et al. 2008) With a difference in mean serum cytokine concentrations of 10 pg/ml we were able to detect a difference with between 81% and 100% power if the standard deviation was between 10 and 20 pg/ml (see Table 3-9).

Epidermal structural and immune biomarkers were also Tier 3 measures. The measurement of interest was whether there were differences in the means of several different biomarkers between groups. In order to estimate the possible differences in means and standard deviations, we used a study by Narendran et al. comparing the biomarkers of epidermal innate immunity in premature and full term infants. (Narendran, Visscher et al. 2010) With a difference in mean epidermal cytokine concentrations between infants in each oil group of 1.5 pg/µg of protein we could detect a difference with between 81% and 100% power if the standard deviation ranged from 1-3 pg/µg of protein (see Table 3-10). We had 81-100% power to detect a difference of 10 pg/µg of protein in mean structural protein concentrations between infants massaged with sunflower oil versus mustard seed oil with standard deviations between 10 and 20 pg/µg of protein (see Table 3-11).

Data Analysis

All analyses for these measurements were conducted using STATA v12 (College Station, TX). The first step in the analysis process was exploratory in order to examine the range of study variables and some of the possible relationships in order to better understand the data. This included tabulations, examination of the means, plotting the distributions, box plots, and scatter plots. The comparability of the treatment groups in relation to their background characteristics was also assessed to determine if there were any confounding variables. The possible confounders that were investigated included: household demographics and socioeconomic status (SES), ethnic group, maternal and paternal education levels, maternal age, reproductive history, receipt of interventions, labor and delivery characteristics (place of birth, length and type of labor, type and practice of birth assistants), newborn characteristics and newborn care practices (sex, birthweight, gestational age, breastfeeding), and intervention exposure and compliance. Adjustment for any unbalanced variables occurred during analysis. All statistics were evaluated at a significance level of $p < 0.05$. Sub-group analyses were also conducted for preterm infants (<37 weeks) for each measurement. All analyses followed an intention-to-treat approach for participating infants, regardless of the actual treatment provided.

Specific Aim 1: Skin Integrity and Barrier Function

Descriptive statistics of each outcome of interest were computed. These included means, standard deviations (within and between groups), minimums, and maximums for skin condition scores, TEWL, skin pH, and protein concentration. Outliers that were biologically impossible were removed from analyses on the basis that they were likely due to an instrument malfunction or measurement of another biological process (e.g. measurement of perspiration rather than TEWL). Skin pH values greater than 8 and less than 3 were removed (Marty Visscher, personal communication) as well as TEWL values greater than 100 $g/m^2/hr$. (Aki Immonen, personal communication) A total of 22 (<1%) skin pH measurements were removed, although due to using the mean of 3 measurements, this did not result in losing any observations. Thirty-nine (1.3%) TEWL observations were removed as a result of 133 (4%) measurements being out of range.

In order to analyze differences in skin pH, the differences in the time it took for infants in each group to reach a beneficial skin pH level (<5.7) was assessed using Cox Regression Models. (Yosipovitch, Maayan-Metzger et al. 2000) For this survival analysis, infants were censored at the earliest of: skin pH = 5.7 (event), death, loss to follow-up, and after 28 days.

In order to evaluate skin condition scores, the two scores that measured irritation of the skin (rash and erythema) were added together to get a total score. Due to the low presence of dryness in our study population (only 152 out of 3011 (5%) observations had chest dryness scores that were not zero), dryness scores were not included in our analyses. Individual scores were evaluated for each location (chest, left arm, and right leg). Evaluations were also done on each component of the skin condition score individually for each body region. To examine whether there were differences in mean skin condition scores, TEWL, skin pH, and protein concentration at different follow-up visit times for the different oil groups, mixed-effects regression analyses with random intercepts were performed. In all models, estimates of standard errors accounted for the clustered design. To determine if differences in skin integrity measures were due to the effect of the different oils used, multi-level mixed-effects regression models with random intercepts accounting for the randomized clusters and repeated measures on each infant were performed for the entire neonatal period and stratified by early (<7 days) and late (≥7 days) neonatal periods. Multi-level models with an interaction between infant's age and oil group were also used to explore whether the rate of change in skin pH, TEWL, or protein concentration was different in the sunflower seed oil group during different periods of time. Linear splines were used to account for nonlinearity.

In addition to evaluating whether oil group had an effect on skin integrity measures, further analyses were done to determine risk factors associated with skin barrier measures in this population. Possible risk factors related to the infant, mother, household and environment were evaluated. These included measures of household demographics and SES, ethnic group, maternal and paternal education levels, maternal age, reproductive history, labor and delivery characteristics (place of birth, length and type of labor, type and practice of birth assistants), newborn characteristics and newborn care practices (sex, birthweight, gestational age, breastfeeding, small-for-gestational age (SGA) status), and temperature and humidity measures.

A bivariate analysis was done for each potential risk factor using a mixed-methods approach with random intercepts, accounting for the repeated measures for each infant, to determine whether there was an association between any of the potential risk factors and any of the skin integrity measures. Additionally, a multivariate model, accounting for multiple measures per infant, was constructed from risk factors that were statistically significantly associated with each skin integrity measure in the bivariate analyses and with variables that are believed to be associated with skin integrity in this population, but may not have been statistically significantly associated in the bivariate analyses. Mixed-effects models were also used to assess the relationship between time and skin integrity measures. These analyses were done with linear splines where indicated to account for nonlinearity.

Specific Aim 2: Nutritional Status

Descriptive statistics of each outcome of interest were computed. These included means, standard deviations (within and between groups), minimums, and maximums for weight at the birth assessment visit on day 1 and at follow-up visits on days 3, 7, 10, 14, 21, and 28 and length at the birth assessment visit on day 1 and at follow-up visit 28. The mean change in weight and length between visits was also calculated. In addition, mean, range, and standard deviations of z-scores at visit 28 and numbers and proportions of infants who were <-2 z-scores weight-for-age and height-for-age were determined. Z-scores were calculated using WHO Anthro (version 3.2.2, January 2011). (WHO 2011)

To examine whether there were differences in mean weight, length, and z-scores for weight-for-age and height-for-age at different follow-up visit times between the different oil groups, mixed-effects regression models with random intercepts were performed, accounting for the clustered design in the standard error estimates. These mixed-effects models were also run to investigate differences in the mean change in weight between visits using 1, 2, and 3 visit increments (e.g. difference between weight at the birth visit and follow-up visit on day 3, the birth visit and the follow-up visit on day 7, etc.) and differences in the mean change in length between the birth visit and the follow-up visit on day 28 between the two oil groups. In addition, multi-level mixed-effects regression models with random intercepts, accounting for the clustered design and the repeated measures in the standard error estimates, were performed to determine if oil group had any effect on weight and length during the entire neonatal period and when stratified by early (<7 days, including the initial birth visit) and late (≥7 days) neonatal periods. Multi-level models with an interaction between infant's age and oil group were also used to explore whether the rate of change in weight or length was different in the sunflower seed oil group during different time periods. Linear splines were used to account for nonlinearity. Mixed-effects logistic regression models with random-intercepts, accounting for the clustered design in the standard error estimates, were run to determine if there were differences in the proportion of infants who were stunted or underweight (<2 z-scores below normal) at 28 days of age. Sub-group analyses were conducted for preterm infants and SGA status for each measurement.

Specific Aim 3: Microbial Challenge and Specific Aim 4: Systemic and Epidermal Innate Immunity

As the results of these specific aims are not reported in this dissertation, their statistical analyses are also not discussed.

Chapter 3 Tables and Figures

Table 3-1: Scheduling of Visits and Associated Data Items Collected for Mechanisms Study

Measure	# of Infants	1	3	7	10	14	21	28	180
Activities for Birth Team Members									
Sampling/LMP	1000	X							
Birth Assessment	1000	X							
Length	1000	X						X	X
Weight	1000	X	X	X	X	X	X	X	X
Activities for Biological Mechanisms Team-No Cold Chain Required									
Consent	1000	X							
Skin Condition Score	1000	X	X	X		X		X	
TEWL	1000	X	X	X		X		X	
Skin pH	1000	X	X	X		X		X	
Activities for Biological Mechanisms Team-Cold Chain Required									
Skin Discs	1000	X		X		X		X	
Saliva	1000	X		X		X			
Skin Flora	400	X	X	X					
Heelprick	200					X			

Table 3-2: VDCs Participating in NNIPS Oil Massage Study and Mechanisms Study

VDC Number	VDC Name	Participated in Mechanisms Study
08	Murtiya	Yes
11	Barahathawa	Yes
12	Laukat	Yes
13	Sundarpur	Yes
14	Manipur	Yes
15	Harkathawa	No
16	Bhawanipur	No
17	Dhankaul	No
30	Janakinagar	Yes
31	Mainathpur	No
32	Dhangada	Yes
33	Sahodawa	Yes
43	Sisautiya	Yes

Table 3-3: Skin Condition Score Scale

	Severity	Area
Erythema Scale		
0	None	None
1	Faint or definite pink	<2%
2	Definite red	2-10%
3	Very intense redness	10-50%
4	NA	>50%
Rash Scale		
0	None	None
1	Papules	One
2	Pustules	2-5
3	Papules and pustules	<10%
4	Clear fluid-filled vesicles	10-50%
5	NA	>50%
6	NA	Numerous and continuous/joining
Dryness Scale		
0	None	None
1	Slight powderiness	<10%
2	Early cracking	10-50%
3	Moderate cracking & scales	>50%
4	High cracking & lifting scales	NA
5	Bleeding cracks	NA

NA=Not applicable

Table 3-4: Power to Observe a Difference in Means of Skin Integrity Measures (500 infants in each oil group)

Standard Deviation	Difference in means at each visit of infants massaged with sunflower oil versus mustard seed oil		
	0.25	0.5	0.75
1.0	88	100	100
1.5	56	99	100
2.0	35	88	100
2.5	24	71	97

Table 3-5: Power to Observe a Difference in Proportion of Newborns Stunted or Underweight (500 infants in each oil group)

Absolute Difference	Proportion of infants massaged with mustard seed oil with <-2 SD below the normal mean weight-for-age or height-for-age				
	.1	.2	.3	.4	.5
0.05	61	34	26	29	22
0.10	100	93	81	73	69
0.15	100	100	99	98	96
0.20	100	100	100	100	100

Table 3-6: Power to Observe a Difference in Mean Percentage of Fatty Acids that is Linoleic Acid (100 infants in each oil group)

Standard Deviation (%)	Difference in mean percentage of fatty acids that is linoleic acid between infants massaged with sunflower oil versus mustard seed oil (%)		
	5	10	15
5	100	100	100
10	81	100	100
15	47	97	100
20	29	81	99

Table 3-7: Power to Observe a Difference in Mean Amounts of Skin Flora (200 infants in each oil group)

Standard Deviation (log_{10}CFU/ml)	Difference in mean bacterial colonization of infants massaged with sunflower oil versus mustard seed oil (log_{10}CFU/ml)				
	0.1	0.2	0.3	0.4	0.5
0.5	36	89	100	100	100
1.0	12	36	67	89	98
1.5	8	19	36	57	76
2.0	6	12	22	36	51

Table 3-8: Power to Observe a Difference in Mean Salivary Cytokine Concentrations (200 infants in each oil group)

Standard Deviation (pg/ml)	Difference in mean salivary cytokine concentrations in saliva between infants massaged with sunflower oil versus mustard seed oil (pg/ml)		
	5	10	15
10	98	100	100
15	76	100	100
20	51	98	100
25	36	89	100

Table 3-9: Power to Observe a Difference in Mean Serum Cytokine Concentrations (100 infants in each oil group)

Standard Deviation (pg/ml)	Difference in mean serum cytokine concentrations between infants massaged with sunflower oil versus mustard seed oil (pg/ml)		
	5	10	15
10	81	100	100
15	47	97	100
20	29	81	99
25	21	62	92

Table 3-10: Power to Observe a Difference in Mean Epidermal Cytokine Concentrations (100 infants in each oil group)

Standard Deviation (pg/μg of protein)	Difference in mean epidermal cytokine concentrations between infants massaged with sunflower oil versus mustard seed oil (pg/μg of protein)				
	0.5	1	1.5	2	2.5
1	81	100	100	100	100
2	29	81	99	100	100
3	16	47	81	97	99
4	11	29	56	81	94

Table 3-11: Power to Observe a Difference in Mean Structural Protein Concentrations (100 infants in each oil group)

Standard Deviation (pg/μg of protein)	Difference in mean structural protein concentrations between infants massaged with sunflower oil versus mustard seed oil (pg/μg of protein)		
	5	10	15
10	81	100	100
15	47	97	100
20	29	81	99
25	21	62	92

Figure 3-1: NOMS and Oil Mechanisms Study VDCs

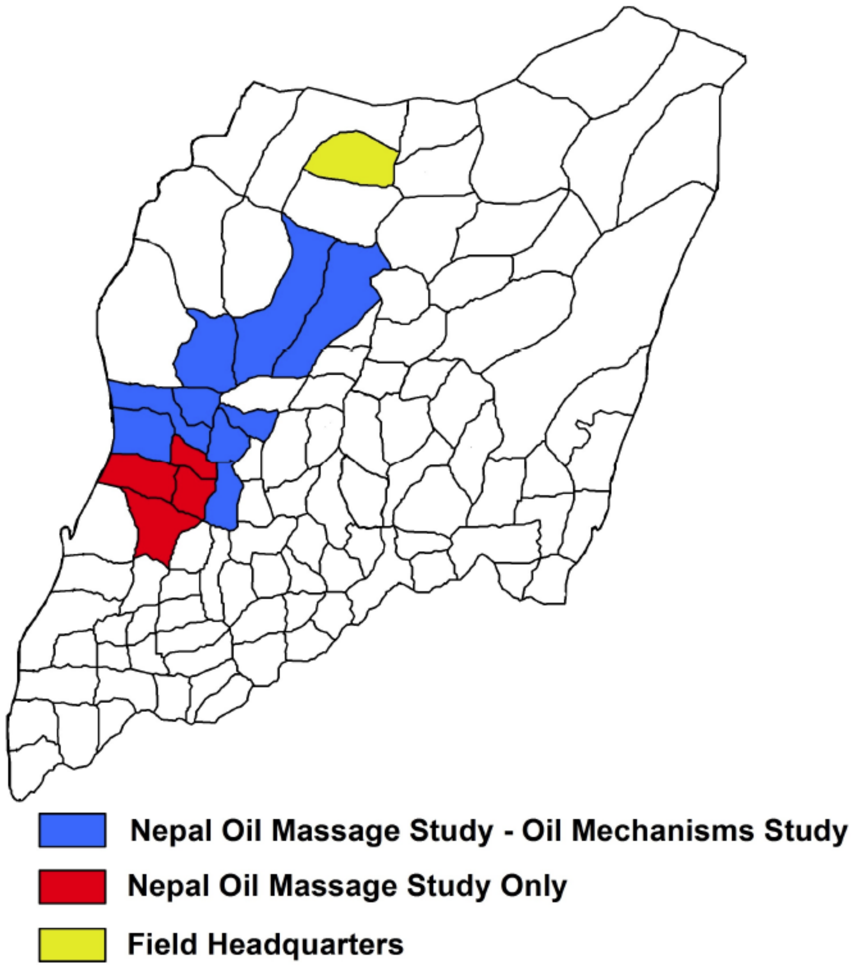


Figure 3-2: Flowchart of NOMS Trial Study Design

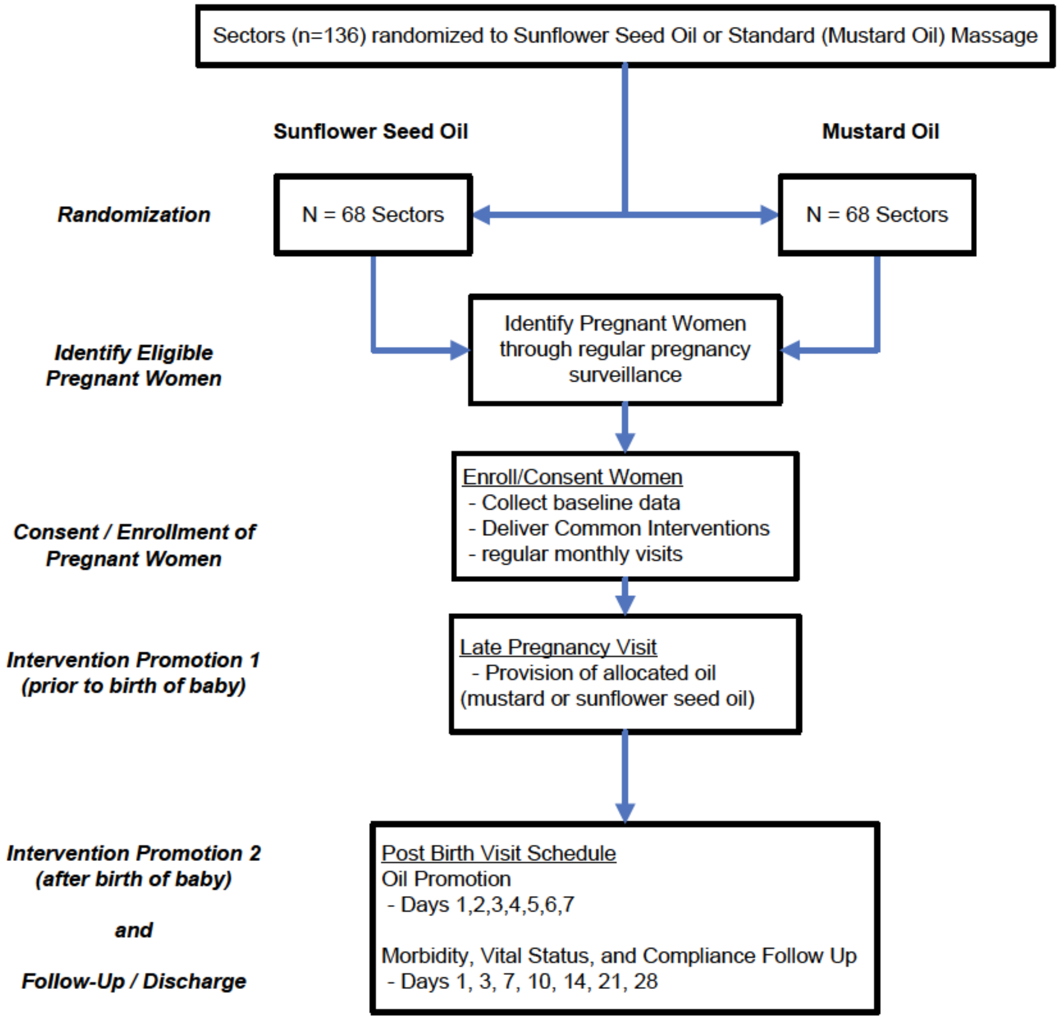
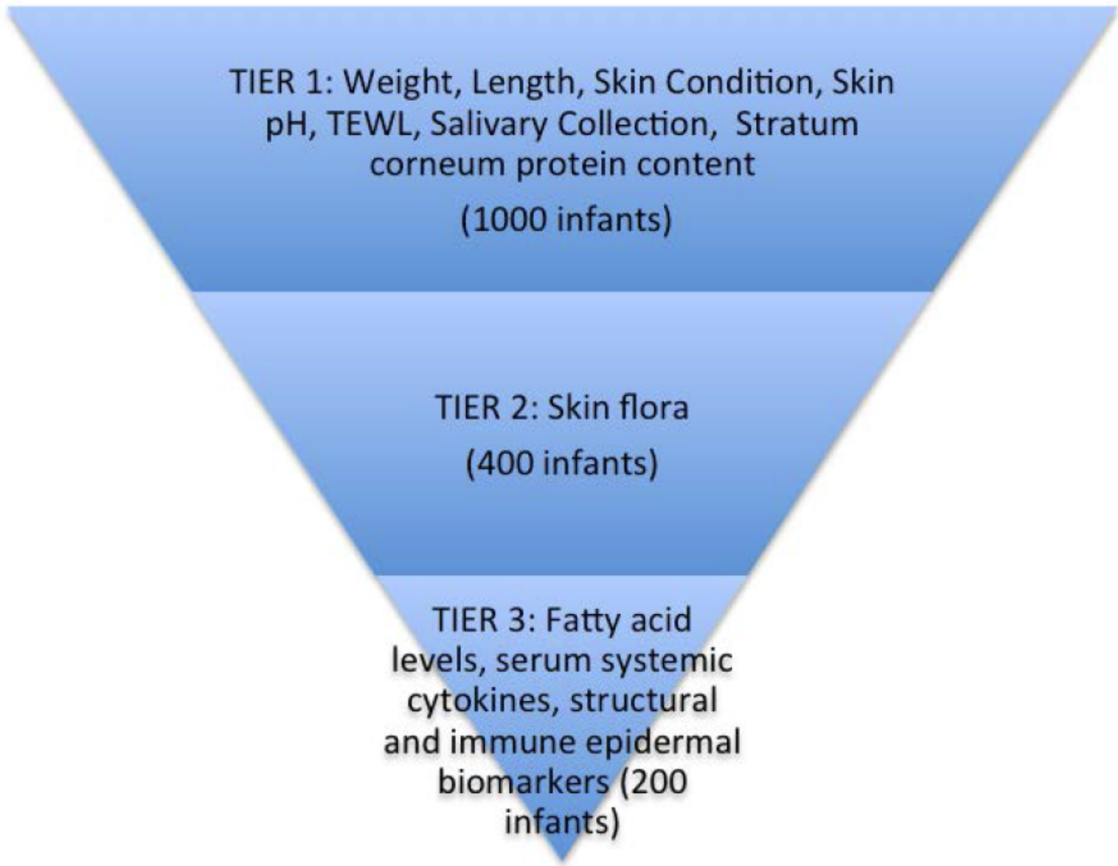


Figure 3-3: Structure of Sample Sizes for Biological Mechanisms Study Measurements



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Chapter 4 Epidemiology and Risk Factors Associated with Skin Integrity and Barrier Function

Background

Newborn skin is an important organ protecting the neonate, serving as the first line of defense between the infant and the environment, the stratum corneum being where most of this important barrier function occurs. The skin serves many important functions including: moderating fluctuations in transepidermal water loss (TEWL) and maintaining electrolyte homeostasis, thermoregulation and minimizing caloric losses, antimicrobial defense, protection from environmental toxins, protection from ultraviolet (UV) radiation, and tactile stimulation. (Darmstadt and Dinulos 2000)

The skin is well developed and fully functional at birth in full term infants, with a thick epidermis and well-formed stratum corneum layers, despite being exposed to water and amniotic fluid for nine months. (Cunico, Maibach et al. 1977; Yosipovitch, Maayan-Metzger et al. 2000) Epidermal barrier properties such as increased hydration and water binding undergo progressive changes during a healthy full term infant's first month of life as it adapts to the dry extrauterine environment. (Visscher, Chatterjee et al. 2000) Neonatal skin is always adjusting to its extrauterine environment when comparing parameters such as skin thickness, skin pH, TEWL, lipid content, and stratum corneum hydration to adult skin, which is in a steady state, suggesting a balance between different parameters may be important for barrier function. (Chiou and Blume-Peytavi 2004; Fluhr, Darlenski et al. 2012; King, Balaji et al. 2013) In addition, water handling behaviors and levels of stratum corneum water binding amino acids continue to develop during the first year of life. (Nikolovski, Stamatas et al. 2008)

Preterm infants show immature barrier function, including: structural immaturity (i.e. the stratum corneum and epidermis are thinner leading to increased susceptibility to shear forces), an underdeveloped stratum corneum epidermal permeability barrier (leading to increased TEWL, loss of heat, increased caloric demands, an increase in potential for environmental toxin absorption, and compromised antimicrobial defense), slower formation of the antibacterial acid mantel, incomplete development of vernix (and subsequent effects), and increased UV susceptibility. (Evans and Rutter 1986; Cartlidge 2000; Darmstadt and Dinulos 2000; Rutter 2000) In addition, neonates, especially those who are preterm, are at high risk for infection, which could be due in part to their epidermal barrier immaturity (including fewer stratum corneum layers, poorly formed lipid layers, and increased permeability). (Nickoloff and Naidu 1994; Nishijima, Tokura et al. 1997)

An important function of the skin barrier is to protect against transepidermal water loss, a direct function of gestational age. (Hoath and Maibach 2003) TEWL measures in full term infants are very low at birth, equal to or lower than adult values, indicating a highly effective skin barrier. (Cunico, Maibach et al. 1977; Yosipovitch, Maayan-Metzger et al. 2000) A reference dataset of over 1000 term neonates had a mean TEWL of 7.06 $g/m^2/hr$. (Kelleher, O'Carroll et al. 2013) In contrast, very low birthweight preterm infants have very high TEWL when measured at birth, ranging from 50 to 70 $g/m^2/hr$. (Hammarlund and Sedin 1979) In addition to gestational age, TEWL is also dependent on the site of measurement, ambient humidity, temperature, activity, and nutritional status at birth. (Oberg, Hammarlund et al. 1981; Hammarlund, Sedin et al. 1982; Hammarlund, Sedin et al. 1983)

The acidic pH of the skin is another mode of protection. This acidic surface of the skin is important in the formation and integrity of the stratum corneum. It allows the effective functioning of enzymes that are involved in lipid metabolism, bilayer structure, ceramide synthesis, and desquamatization. (Rippke, Schreiner et al. 2002; Schmid-Wendtner and Korting 2006) After birth, skin pH is elevated in full term neonates compared with adults and older children and decreases in the first few weeks, most significantly during the first 4 days. (Green, Carol et al. 1968; Fluhr, Pfisterer et al. 2000; Visscher, Chatterjee et al. 2000; Hoeger and Enzmann 2002). The mean skin pH of full term neonates within the first 10 hours after birth from six different body sites was 7.08 compared to a mean of 5.7 in adults. (Yosipovitch, Maayan-Metzger et al. 2000; Hoeger and Enzmann 2002) Some studies have suggested development of the acid mantle is not correlated with gestational age, with development in preterm neonates shown to be very close to that of full term neonates. (Green, Carol et al. 1968) However, other studies have shown that for very preterm infants, skin pH tends to remain higher for a longer period of time. (Fox, Nelson et al. 1998)

Recent observations of reductions in nosocomial infections and mortality among preterm infants receiving topical applications of sunflower seed oil (Darmstadt, Badrawi et al. 2004; Darmstadt, Saha et al. 2005; Darmstadt, Saha et al. 2008) have been posited as possibly attributable to improvement in skin barrier function in the exposed infants. The epidemiological results have also prompted large-scale community based studies (such as ours) of the impact of substituting sunflower seed oil in place of the more commonly practiced application of mustard oil. However, while changes in skin barrier integrity and function could be one of the ways in which emollient therapy with vegetable oil may improve the health of newborns, little is known about the risk factors associated with differences in skin barrier integrity in many of the communities that routinely practice neonatal oil massage with locally available vegetable oils. We aimed to determine possible risk factors associated with skin barrier integrity and skin condition, measured visually as rash and erythema, and instrumentally as skin pH, TEWL, and stratum corneum protein concentrations in such a population in rural Nepal. An examination of these risk factors will be important in understanding how neonatal oil massage may influence skin barrier integrity and possibly contribute to improved health of newborns.

Methods

Settings and Population

The data for this study were collected as part of a cluster-randomized community-based trial conducted by the Nepal Nutrition Intervention Project, Sarlahi (NNIPS). In 2011, the NNIPS surveillance area consisted of 26 Village Development Committees (VDCs) each encompassing nine government defined geopolitical units (wards), which were further divided into sectors based on population. This trial was nested within a larger parent trial on the Impact of Sunflower Seed Oil Massage on Neonatal Morbidity and Mortality in Nepal (NOMS). The study population for the NOMS trial was all live born infants in households in 13 VDCs in Sarlahi District in rural Nepal. The Nepal Health Research Council and the Committee on Human Research of the Johns Hopkins Bloomberg School of Public Health approved this study.

Procedures and Design of the Parent Trial

Previous sector level mortality estimates were known for 9 of the 13 VDCs from prior research in this community and were used to perform restricted randomization in order to ensure balance of prior neonatal mortality risk. In the other 4 VDCs, sectors (clusters) were randomized with a computerized quasi-random number generator, stratified on

VDC using blocks of 4, ensuring a geographical balance of the types of oil across the study area. Newborn infants were randomized within clusters (sectors) to receive either promotion of full body massage with sunflower seed oil or promotion of full body massage with mustard seed oil. A cluster-randomized design was chosen in order to minimize the chance of crossover or contamination of the intervention by providing each field worker with only one type of oil to promote in her area. It was not possible to blind field workers or mothers to the treatment, as mustard oil and sunflower oil have distinct colors and smells.

Community-specific lists of married women of reproductive age were created. In order to rapidly identify new pregnancies, locally-resident female workers monitored the status of these women every 5 weeks, asking about menstruation in the prior month, and conducting pregnancy tests. A woman identified as pregnant was approached for recruitment and consent for her infant's participation in the parent trial. Each woman participating in the study received a set of basic antenatal care interventions (e.g. tetanus toxoid, a clean delivery kit, iron-folate supplements, chlorhexidine cleaning solution for disinfecting the umbilical region, and deworming), along with basic educational messages on antenatal and newborn care. The worker visited the women in late pregnancy (~28-32 weeks) to promote the use of either the sunflower seed oil or mustard seed oil, and provide the mother or other caretaker with a 100ml bottle of oil at that time. The mother or other caretaker initiated full body massage following the field worker's guidelines using the provided oil daily throughout the newborn period. During the first week of life, the local worker visited the homes daily to promote the continued use of the oil.

Immediately upon notification of the pregnancy, the local resident worker notified the supervisory staff and a birth assessment was conducted using standardized data collection forms recording information related to late pregnancy morbidity, labor and delivery characteristics, birth assistants and practices, length of labor, maternal temperature, date and time of birth, sex, length and weight of infants, and immediate newborn care practices (thermal care, cord practices, early bathing and massage, breastfeeding initiation, etc.). In addition to the individual data obtained, household level data were collected at the time of enrollment of the mothers. This included information on socioeconomic factors, such as ethnicity, caste, household assets, ownership of materials or livestock, water sources, and family members who may be working overseas. Data were also collected on parental literacy, education, and birth history. After the initial birth visit, the birth team member completed newborn follow-up visits (NFF) on days 3, 7, 10, 14, 21, and 28 (for a total of 7 visits), where signs of infant morbidity and newborn care practices since prior visit were recorded. A 500ml bottle of oil was provided at the initial visit and on follow-up visits on days 10 and 21. The primary outcomes of NOMS are neonatal mortality and possible severe infections.

Design of the Biological Mechanisms Sub-Study

This biological mechanisms sub-study included an extended set of measurements collected from a subset of infants participating in the main trial. The focus of data collection for this sub-study was on direct measurement of biological markers of infants built into a specific sub-study schedule (days 1, 3, 7, 14, and 28) of the parent trial visits. The biological measurements used to assess skin integrity included: visual assessment of skin condition (erythema, rash), measurement of transepidermal water loss (TEWL), measurement of skin pH, and collection of skin discs in order to measure the stratum corneum cohesion and protein concentration via measurement of optical density. All skin integrity measures were taken at each of the five visits, apart from the skin disc collection, which was collected at visits 1, 7, 14, and 28. Table 4-1 shows the timing of measurement collections.

A subset of 7 VDCs of the 13 original VDCs in the NOMS trial was selected to participate in the mechanisms study, which began in July 2012. An 8th VDC was added after 5 months and a 9th VDC was added in January 2013. The cluster-randomization of the parent trial was conserved for the mechanisms study. Among infants participating in the main study, determination of eligibility to additionally participate in the sub-study was done during the initial birth assessment visit, and was based on estimates of gestational age at birth. For this study, preterm infants (<37 weeks gestational age) were oversampled, aiming for approximately 50% of the sample to be preterm. During the study period (July 2012 until September 2013), the gestational age was estimated directly by the field worker using the date of last menstrual period estimated by the woman at the time of initial enrollment. Each birth team member had a list of the date of the mother's last menstrual period (LMP), which was used along with the date of birth to determine the gestational age of the infant. If the infant was born before week 37, one of the members of a specially trained team of field workers focused on the implementation of the mechanisms sub-study was contacted directly by mobile phone. Infants born on or after week 37 were listed by VDC in consecutive order (i.e. by birth date) on a pre-printed computer generated blank form of 20 rows, 4 of which had been randomly selected for shading. Infants listed on a randomly shaded row were eligible for inclusion in the mechanisms study, thus selecting 4 out of every 20 (20%) of the full term infants for participation. All preterm (<37 weeks) infants and every 5th full term infant born in the mechanisms study area were eligible for enrollment in the sub-study. Infants were enrolled if consent was provided and the infant was met alive within 48 hours after birth.

Newborns enrolled in the mechanisms study were visited in their homes 5 times, on days 1, 3, 7, 14, and 28. In addition, to performing the skin integrity measurements at these visits, the workers asked questions relating to newborn care practices since the prior visit, including breastfeeding and other feeding practices, bathing, and massage practices.

Measurement Methods

Four measures were taken to evaluate skin integrity: skin condition scores, transepidermal water loss (TEWL), skin pH, and protein concentrations (via optical density). Field worker evaluation of skin condition was measured using a modified version of a scoring method described by Visscher et al. (Visscher, Odio et al. 2009) This team has experience extending these scores for patients in the United States. This study used an extended version of more established scales for the measure of irritation of the skin based on levels of erythema and rash, rating erythema severity on a scale from 0 to 3 and area affected on a scale from 0 to 4. Severity of rash and area affected by rash were evaluated on scales from 0 to 4 and 0 to 6 respectively. Scores were based on the percent area of involvement and severity of skin compromised within the specified body region for each component. Each field worker was trained in the use of the scales. The severity of erythema and rash as well as the area covered was recorded on standardized data collection instruments. Skin condition assessments were completed on the same 4cm by 4cm area of the chest, the entire left arm, and the entire right leg.

Transepidermal water loss was measured in $g/m^2/hr$ using a VapoMeter (Delfin Technologies, Ltd, Finland), a closed-chamber device containing sensors for relative humidity and temperature, using standardized procedures. (Rogeries and Group 2001; Nuutinen, Alanen et al. 2003; De Paepe, Houben et al. 2005) TEWL measurements were taken three times at the mid-chest nipple line. The mean value of these measurements was used for analyses. The exact location where measurements were taken was similar in all infants, through the measurement of anatomical markers. The location of the measurements was chosen because it was easily accessible, an area that was massaged, and provided a reasonably flat surface area for placing the VapoMeter. The three measurements along with the temperature and relative humidity obtained from the VapoMeter were recorded on standardized data collection forms.

Measurements of skin pH were performed with a flat electrode (Skincheck™, Hanna Instruments, UK) calibrated daily to pH 4 and 7. (Parra and Paye 2003) Measurements were taken in the same location at the mid-nipple as the TEWL measurements. The three measurements were recorded on standardized data collection forms and the mean skin pH of the three measurements was used for analyses.

Using a technique described by Voeglei et al., protein content from the outermost stratum corneum was analyzed using samples collected using 380 mm^2 D-Squame adhesive discs (CuDerm, TX) applied to the chest with constant pressure for 2 minutes. (Voegeli, Heiland et al. 2007) Different locations on the chest were used for the placement of the skin discs at each of the four visits in order to collect discs from skin that had not been previously exposed to adhesive. These locations were different from those used to measure TEWL and skin pH. After the discs were removed from the chest, they were placed in microtubes and transferred to field headquarters in cold boxes kept between 2° and 8°C. At field headquarters, optical absorption of the skin discs was determined using a spectrophotometer SquameScan™ 850A (Heiland electronic, Wetzlar, Germany) specifically designed for the application of D-Squame discs. Before the optical absorptions of the discs were read, the spectrophotometer was calibrated to

0% and 36.2% optical absorption. Optical absorption of the discs was recorded on standardized data collection forms. The following equation was used for quantification of protein concentration: (Voegeli, Rawlings et al. 2007)

$$C_{protein}\left(\frac{\mu g}{cm^2}\right) = 1.366 * Absorption(\%) - 1.557$$

Statistical Analysis

The nested biological mechanisms study included a subset of the sample size of 29,620 infants required for the parent trial. Given that we hypothesized that the outcome measures may be more important in preterm infants than full term infants due to the immaturity of their skin, preterm infants were oversampled. Due to the limited number of instruments (VapoMeters, pH meters) available for multiple field sites, a total sample size of 1000 infants was selected, equally stratified by 500 preterm and 500 full term infants, in order to obtain the power needed to detect any possible effects of the different oil groups on skin integrity measurements. The analyses presented here represent a preliminary analysis of the first 63.7% of the anticipated enrolled infants.

All analyses for these measurements were conducted using STATA v12 (College Station, TX). Possible risk factors related to the infant, mother, household and environment that may be associated with skin integrity measures were evaluated. These included measures of household demographics and socioeconomic status (SES), ethnic group, maternal and paternal education levels, maternal age, reproductive history, labor and delivery characteristics (place of birth, length and type of labor, type and practice of birth assistants), newborn characteristics and newborn care practices (sex, birthweight, gestational age, breastfeeding, small-for-gestational age (SGA) status), and temperature and humidity measures. Outliers that were biologically impossible were removed from the analyses on the basis that they were likely due to an instrument malfunction or measurement of another biological process (e.g. measurement of perspiration rather than TEWL). Skin pH values greater than 8 and less than 3 were removed (Marty Visscher, personal communication) as well as TEWL values greater than 100 $g/m^2/hr$. (Aki Immonen, personal communication) A total of 22 (<1%) skin pH measurements were removed, although due to using the mean of 3 measurements, this did not result in any lost observations. Thirty-nine (1.3%) TEWL observations were removed as a result of 133 (4%) measurements being out of range.

A bivariate analysis was done for each potential risk factor using a mixed-methods approach with random intercepts, accounting for the repeated measures on each infant in the estimates of standard errors, to determine whether there was an association between any of the potential risk factors and any of the skin integrity measures. Additionally, a multivariate model, accounting for multiple measures per infant, was constructed from risk factors that were statistically significantly associated with each skin integrity measure in the bivariate analyses and with variables that are believed to be associated with skin integrity in this population, but may not have been statistically significantly associated in the bivariate analyses. Co-linear variables were not included in the multivariate model. Mixed-effects models were also used to assess the relationship between time and skin integrity measures. Linear splines for infant's age at measurement were used where needed to account for nonlinearity, assessed through locally weighted regression smoothing.

Results

Between July 23, 2012 and September 30, 2013, there were a total of 1,876 full term and 409 preterm live born infants in the study area that were eligible for enrollment in the biological mechanisms study. Of these infants, 785 were selected for enrollment to date (Figure 4-1). Of those selected, 148 (18.9%) died or were not met by the mechanisms study field worker before 48 hours after birth and 5 (0.6%) mothers refused their infant's participation in the study. A total of 632 newborns were enrolled, with 533 (84.3%) completing 28 days of follow-up. A total of 13 (2%) neonatal deaths occurred and 86 (13.6%) infants moved before the 28 days were competed, most likely because the mother was returning to her husband's house after giving birth at her maternal home or *maiti*.

Infants' background characteristics are shown in Table 4-2. Sex was fairly balanced with 51.6% of the study population male and 48.4% of the population female. The mean gestational age of infants in this study was 37.7 (±3.6) weeks, however preterm infants were oversampled, so this is not representative of births in the population. Mean weight at the initial visit was 2611.8g (±483.8). Infants in this population are massaged with oil quite frequently. The average number of massages infants received per day during the first week of life was 4.5 (±1.4).

Socioeconomic, household, and maternal characteristics are shown in Table 4-3. The majority of infants in this population were of *Madeshi* ethnicity (92.8%), compared with only 7.2% *Pahadi*. The majority of mothers gave birth in the home (71.5%) and only 32.4% of women had a skilled attendant at delivery. In addition, 29.2% of women did not receive any antenatal care during pregnancy. Most women in this population had not received any education (71.7%) and only 28.5% of mothers were literate.

Table 4-4 shows some of the environmental characteristics during this study period. Only 8.3% of visits took place when the relative humidity was <60%. The mean temperature at visits during the study period was 28.7°C (±4.2) and the average relative humidity was 77.8% (±11.7). The average heat index, which is a function of the temperature and relative humidity, was 35.9°C (±9.0), and heat index exceeded 35°C on 56% of study days.

Risk Factors Related to TEWL

There is an overall trend of increasing TEWL during the neonatal period for infants in this study, with the fastest rate of increase occurring between days 0 and 3 (Figure 4-2). At visit 1, infants have a mean TEWL of 34.8 $g/m^2/hr$ (±22.9) and by visit 28 the mean TEWL increased to 43.3 $g/m^2/hr$ (±23.2) (Table 4-5). Between days 0 and 3, infants' TEWL increased an estimated 1.71 $g/m^2/hr$ (95% CI: 0.86-2.55) per day (Table 4-6). Between days 4 and 28 TEWL continued increasing, but at a slower rate of 0.22 $g/m^2/hr$ (95% CI: 0.13-0.30) per day. Table 4-6 shows the possible risk factors of infant characteristics that may be associated with TEWL in this population. These bivariate analyses indicate that in addition to age, sex and hours before breastfeeding initiation were statistically significantly associated with TEWL. The results estimate that females had a TEWL value 3.77 $g/m^2/hr$ (95% CI: 1.09-6.44) lower than males. The results also indicated that infants who were first breastfed 5 or more hours after birth had TEWL values 6.44 $g/m^2/hr$ (95% CI: 0.21-12.68) higher compared with infants who were first breastfed less than 1 hour after birth. In this population, gestational age was not statistically significantly associated with differences in TEWL, however there was a trend of increasing TEWL with increasing gestational age.

The bivariate analyses of variables associated with the mother and the household are shown in Table 4-7. These results indicate maternal literacy, whether the household had electricity, and whether the mother received antenatal care were statistically significantly associated with changes in TEWL. The results estimate infants whose mother is literate have 3.40 $g/m^2/hr$ (95% CI: 0.43-6.37) higher values of TEWL than infants whose mothers are illiterate. In addition, infants whose household had electricity had 3.71 $g/m^2/hr$ (95% CI: 0.96-6.47) higher values of TEWL when compared with infants whose households did not have electricity. Infants whose mothers attended antenatal care visits also had increased TEWL when compared with infants whose mothers did not receive any antenatal care, with infants whose mothers attended 1-2 antenatal care visits having estimated TEWL measures 5.49 $g/m^2/hr$ (95% CI: 2.18-8.80) higher and infants whose mothers attended 3 or more antenatal care visits having higher TEWL of 5.28 $g/m^2/hr$ (95% CI: 1.93-8.64).

Table 4-8 shows the results of bivariate analyses of variables related to the environment and their impact on TEWL. These results indicate temperature, humidity, and heat index are all statistically significantly associated with TEWL, with humidity associated with increases in TEWL and temperature and heat index associated with decreases. For each 10°C increase in temperature, TEWL is estimated to decrease 10.62 $g/m^2/hr$ (95% CI: 10.38-10.86). Similarly, with each 10°C increase in heat index, TEWL is estimated to decrease 4.9 $g/m^2/hr$ (95% CI: 3.7-6.1). With each 10% increase in relative humidity, TEWL is estimated to increase 5.1 $g/m^2/hr$ (95% CI: 4.3-5.9). Comparing infants' TEWL values where the relative humidity at the time of TEWL measure was >80% with infants' TEWL values where the relative humidity at the time of TEWL measure was <40%, TEWL values were 13.95 $g/m^2/hr$ (95% CI: 6.28-21.62) higher.

A multivariate model, adjusted for multiple measures per infant, was constructed from variables that were statistically significantly associated with TEWL in the bivariate analyses and with variables that may not have been statically significantly associated in the bivariate models but are believed to be associated with TEWL in this population. This model is shown in Table 4-9. The variables that were included in the multivariate model were: preterm status, sex, ethnicity (whether they were *Pahadi* or *Madeshi*), maternal literacy, whether the household had electricity, antenatal care, time from birth to breastfeeding initiation, temperature, relative humidity, and infant's age from days 0 to 3 and 4 to 28 (using linear splines to account for nonlinearity). Unlike in the bivariate analyses, in the multivariate model, there were no statistically significant associations between maternal literacy or whether a household had electricity with TEWL. In addition, there were no statistically significant associations between ethnicity and term status on TEWL. In this model, female infants were estimated to have a TEWL 2.99 g/m^2/hr (95% CI: 0.40-5.57) lower than male infants. Infants whose mothers attended 1-2 antenatal care visits had increased TEWL of 4.68 g/m^2/hr (95% CI: 1.42-7.94) when compared with infants whose mothers did not receive any antenatal care. Infants whose mothers attended 3 or more antenatal care visits had estimated TEWL measures 3.87 g/m^2/hr (95% CI: 0.53-7.22) higher when compared with infants whose mothers received no antenatal care. Infants who had breastfeeding initiated 5 or more hours after birth had an estimated increase in TEWL measures of 5.89 g/m^2/hr (95% CI: 0.29-11.49) compared with infants who had breastfeeding initiated within 1 hour. When controlling for other variables in the model, for each increase in temperature of 10°C, TEWL was estimated to decrease 12.4 g/m^2/hr (95% CI: 9.7-15.0) and with each increase of 10% relative humidity, TEWL was estimated to increase 3.6 g/m^2/hr (95% CI: 2.8-4.5). In the multivariate model, TEWL increased an estimated 0.96 g/m^2/hr (95% CI: 0.05-1.87) per day between days 0 and 3 and 0.20 $g/m^2/hr$ (95% CI: 0.11-0.29) per day between days 4 and 28.

Risk Factors Related to Skin pH

The relationship of skin pH with age during the neonatal period is shown in Figure 4-3. There is a decrease in skin pH throughout the neonatal period, with a greater rate of decrease during the first 7 days of life (estimated to be 0.14 (95% CI: 0.13-0.14) per day), and a slower rate of decrease from days 8 to 28 (estimated to be 0.016 (95% CI: 0.014-0.018) per day) (Table 4-10). Infants in this population have an estimated mean skin pH of 6.03 (±0.52) and 4.82 (±0.57) at visits 1 and 28 respectively (Table 4-5). Possible infant characteristics that may be associated with skin pH in this population are shown in Table 4-10. The results from the bivariate analyses indicate that along with age, both birthweight and time between follow-up visit and last massage were statistically significantly associated with skin pH. The results estimate that low birthweight infants (<2500g) had mean skin pH values 0.089 (95% CI: 0.032-0.15) higher than infants who were not low birthweight. For each 100g increase in first weight measurement at the initial birth assessment, infants' skin pH values decreased an estimated 0.011 (95% CI: 0.005 -0.017). Infants who had a longer interval between the follow-up visit and time since last massaged tended to have lower skin pH values than infants who had a shorter interval. Infants who were last massaged between 120 and 179 minutes before the follow-up visit had a mean skin pH 0.089 (95% CI: 0.0037-0.18) lower compared with infants who were massaged less than 30 minutes before the visit. Infants massaged more than 180 minutes before the follow-up visit had skin pH values 0.23 (95% CI: 0.16-0.30) lower than infants massaged less than 30 minutes before the visit.

Table 4-11 shows the bivariate analyses results of variables associated with the mother and the household. These results indicate ethnicity (*Madeshi* or *Pahadi*), maternal and paternal literacy, maternal and paternal education, number of household assets, whether the household had electricity, facility delivery, and skilled attendant at delivery were statistically significantly associated with changes in skin pH. Infants whose family is *Madeshi* are estimated to have a skin pH 0.15 (95% CI: 0.043-0.26) higher compared with infants whose family is *Pahadi*. The results indicate that infants who have one or both parents who are educated or literate have lower skin pH values than infants whose parents are not. Infants whose mother is literate have 0.080 (95% CI: 0.017-0.14) lower values of skin pH than infants whose mothers are illiterate. Similarly, infants whose father is literate have estimated skin pH values 0.082 (95% CI: 0.025-0.14) lower than infants whose fathers are illiterate. In addition, infants whose mothers have between 1 and 5 years of education have skin pH values 0.10 (95% CI: 0.0011-0.20) lower than infants whose mothers have no education and infants whose fathers have between 6 and 10 years of education have skin pH values 0.11 (95% CI: 0.045-0.17) lower than infants whose fathers have no education. Infants whose households were of higher socioeconomic status (measured by number of household assets and whether the household had electricity) also tended to have lower skin pH values. Infants whose household had electricity had skin pH values 0.077 (95% CI: 0.019-0.13) lower compared with infants whose households did not have electricity. Similarly, infants whose families had between 6 and 10 household assets and more than 10 household assets had skin pH values 0.14 (95% CI: 0.026-0.26) and 0.28 (95% CI: 0.066-0.49) lower compared with infants whose households had 0 or 1 asset(s). Infants whose mothers delivered at a facility had estimated skin pH values 0.076 (95% CI: 0.013 -0.14) lower compared with infants whose mothers delivered at home and infants who had a skilled attendant at delivery had estimated skin pH values 0.085 (95% CI: 0.025-0.15) lower compared with infants with no skilled attendant at delivery.

Bivariate analyses showing results of variables related to the environment and their impact on skin pH are shown in Table 4-12. These results indicate that temperature, humidity, and heat index are all statistically significantly associated with skin pH, with increased humidity being associated with higher skin pH values and temperature and heat index being associated with lower skin pH values. For each 10°C increase in temperature, skin pH is estimated to decrease 0.19 (95% CI: 0.13-0.25). Similarly, with each 10°C increase in heat index, skin pH is estimated to decrease 0.05 (95% CI: 0.02-0.08). With each 10% increase in relative humidity, skin pH is estimated to increase 0.078 (95% CI: 0.056-0.10).

A multivariate model was constructed from variables that were statistically significantly associated with skin pH in the bivariate analyses and with variables that may not have been statistically significantly associated in the bivariate analyses but are believed to be associated with skin pH in this population. The variables included in the multivariate model were: preterm status, sex, ethnicity, being low birthweight, maternal literacy, paternal literacy, whether the household had electricity, number of household assets, whether a skilled attendant was present during delivery, the time between the visit and the last massage, temperature, relative humidity, and infant's age in days from day 0 to 7 and from days 8 to 28 (using linear splines to account for nonlinearity). The results from this model are shown in Table 4-13. Mother's and father's education, facility delivery, and heat index were not included due to collinearity with other variables. Unlike in the bivariate analyses, in the multivariate model, there was no statistically significant association between maternal or paternal literacy, whether a household had electricity, number of household assets, whether there was a skilled attendant at delivery, or time from last massage to follow-up visit with skin pH. In addition, there was no statistically significant association between whether an infant was preterm or sex on skin pH. After controlling for other variables in the model, *Madeshi* infants' skin pH was estimated to be 0.13 (95% CI: 0.025-0.25) higher than *Pahadi* infants' skin pH. In the multivariate model, infants who were of low birthweight had skin pH values 0.059 (95% CI: 0.0015-0.12) higher compared with infants who were 2500g or more. In this model, for each 10°C increase in temperature, skin pH was estimated to decrease 0.23 (95% CI: 0.17-0.29) and with each 10% increase in relative humidity, skin pH was estimated to increase 0.057 (95% CI: 0.038-0.075). When controlling for other variables in the model, between days 0 and 7, skin pH is estimated to decrease 0.14 (95% CI: 0.13-0.15) per day. When an infant is between 8 and 28 days old, skin pH is estimated to decrease 0.015 (95% CI: 0.012-0.017) per day.

Risk Factors Related to Stratum Corneum Protein Concentration

The relationship between stratum corneum protein concentration removed using skin discs during the neonatal period is shown in Figure 4-4, with lower stratum corneum protein concentrations removed per day during the first week (indicating an increasingly more cohesive and well developed stratum corneum) followed by a slight increase per day from days 8 to 28. Infants started with a high mean stratum corneum protein concentration of 16.4 μg/cm^2 (±7.9) at visit 1, had decreasing protein concentration until visit 7 (12.9 μg/cm^2 (±6.4)), and had an increased mean at visit 28 of 14.0 μg/cm^2 (±6.1) (Table 4-5). During the first week of life, the estimated amount of protein removed decreased 0.58 μg/cm^2 (95% CI: 0.46-0.70) per day, whereas after day 7 the estimated amount of protein removed increased 0.06 μg/cm^2 (95% CI: 0.02-0.10) per day (Table 4-14).

Additional risk factors of infant characteristics that may be associated with protein concentration in this population are shown in Table 4-14. The results from the bivariate analyses indicate that the only variable related to the infant apart from age that was statistically significantly associated with protein concentration was the time between the visit and the last time the infant was massaged, although smaller infants (preterm and lower birthweight) tended to have greater concentrations of protein removed. Infants with a greater amount of time since their last massage tended to have higher protein concentrations, with infants massaged between 60 and 119 minutes before the follow-up visit having an estimated 1.47 μg/cm^2 (95% CI: 0.54-2.40) higher protein concentrations compared with infants massaged less than 30 minutes before the follow-up visit. In addition, infants massaged between 120 and 179 minutes and infants massaged more than 180 minutes before the follow-up visit had estimated protein concentrations 1.22 μg/cm^2 (95% CI: 0.15-2.30) and 1.78 μg/cm^2 (95% CI: 0.88-2.68) higher respectively, when compared with infants massaged less than 30 minutes before the follow-up visit.

Table 4-15 shows the results of bivariate analyses of variables associated with the mother and the household and stratum corneum protein concentration. These results indicate paternal literacy, paternal education, whether the household had electricity, whether the mother received antenatal care, and length of labor were statistically significantly associated with changes in protein concentration. Infants whose fathers were literate or educated tended to have lower amounts of protein concentrations than infants whose fathers were not. The results estimate infants whose fathers are literate have 0.92 μg/cm^2 (95% CI: 0.23-1.60) lower protein concentrations than infants whose father is illiterate and when compared to infants whose fathers received no education, infants whose fathers received between 6 and 10 years had 1.08 μg/cm^2 (95% CI: 0.29-1.87) lower protein concentrations. In addition, infants whose households had electricity had 0.85 μg/cm^2 (95% CI: 0.15-1.55) lower protein concentrations when compared with infants whose households did not have electricity. When compared with infants whose mothers did not receive any antenatal care, infants whose mothers received antenatal care also tended to have lower protein concentrations, with infants whose mothers attended 1-2 antenatal care visits having protein concentrations 1.07 μg/cm^2 (95% CI: 0.22-1.93) lower. Infants whose mothers were in labor more than 5 hours tended to have lower protein concentrations as well. Infants whose mothers were in labor between 5 and 14 hours had protein concentrations 0.89 μg/cm^2 (95% CI: 0.16-1.62) lower compared with infants whose mothers were in labor less than 5 hours.

Bivariate analyses of variables related to the environment and their impact on protein concentrations, indicated that increasing temperature, humidity, and heat index were all statistically significantly associated with a decrease in protein concentration (Table 4-16). For each 10°C increase in temperature, protein concentration decreased an estimated 2.0 μg/cm^2 (95% CI: 1.3-2.7). Similarly, with each 10°C increase in heat index, protein concentration decreased an estimated 1.4 μg/cm^2 (95% CI: 1.0-1.7). With each 10% increase in relative humidity, a decrease of 0.6 μg/cm^2 (95% CI: 0.4-0.9) in protein concentration is estimated.

The results of a multivariate model constructed from variables that were statistically significantly associated with protein concentration in the bivariate analyses and with variables that may not have been statistically significantly associated in the bivariate analyses but are believed to be associated with stratum corneum protein concentration in this population are shown in Table 4-17. The variables included in the multivariate model were: preterm status, sex, ethnicity, paternal literacy, whether the household had electricity, antenatal care received during pregnancy, length of labor, time between visit and last massage, temperature, relative humidity, and infant's age from days 0 to 7 and days 8 to 28 (using splines to account for nonlinearity). Paternal education and heat index were not included due to collinearity with other variables. Unlike in the bivariate analyses, in the multivariate model, there was no statistically significant association between either number of antenatal care visits or whether a household had electricity with protein concentration. In addition, there was no statistically significant association between sex, ethnicity, or whether an infant was born preterm or full term on stratum corneum protein concentrations. After controlling for other variables in the model, infants whose mothers were in labor more than 5 hours tended to have lower protein concentrations, with infants whose mothers were in labor from 5 to 14 hours having protein concentrations 0.79 μg/cm^2 (95% CI: 0.12-1.46) lower when compared with infants whose mothers were in labor for less than 5 hours. Infants whose fathers were literate had estimated protein concentrations 0.96 μg/cm^2 (95% CI: 0.28-1.64) lower than infants whose fathers were illiterate. Increasing time from last massage continued to show a statistically significant trend of increasing protein concentration in the multivariate model. Infants who were massaged between 60 and 119 minutes before the follow-up visit had estimated protein concentrations 1.55 μg/cm^2 (95% CI: 0.65-2.44) higher compared with infants who were massaged less than 30 minutes before the visit. Those infants who were massaged between 120 and 179 minutes and more than 180 minutes before the visit had protein concentrations 1.43 μg/cm^2 (95% CI: 0.39-2.48) and 1.34 μg/cm^2 (95% CI: 1.46-3.22) higher respectively, when compared with infants massaged less than 30 minutes before the follow-up visit. When controlling for other variables in the model, for each 10°C increase in temperature, protein concentration was estimated to decrease 3.1 μg/cm^2 (95% CI: 2.4-3.8) and with each 10% increase in relative humidity, protein concentration was estimated to decrease 0.84 μg/cm^2 (95% CI: 0.58-1.1). After controlling for other variables, the relationship between infants' age and protein concentration persisted, with protein concentrations estimated to decrease 0.57 μg/cm^2 (95% CI: 0.45-0.69) per day between days 0 and 7 and estimated to increase 0.045 μg/cm^2 (95% CI: 0.0047- 0.085) per day between days 8 and 28.

Risk Factors Related to Skin Condition

Figure 4-5 shows the relationships between erythema and rash skin condition scores measured on the chest region over the neonatal period. As an infant gets older, erythema scores tend to increase (become worse) from days 0 to 3, show no or very little change over days 4 to 14, and then decrease (improve) from days 15 to 28. Infants in this population started with a mean skin erythema score of the chest region of 0.53 (±0.67) at visit 1, which increased to 0.83 (±0.66) at visit 3. Visits 14 and 28 had the same mean skin erythema scores as visits 3 and 1 respectively (Table 4-5). Between days 0 and 3 erythema scores showed an estimated increase of 0.14 (95% CI: 0.11-0.17) per day. During the period between days 4 and 14, there was no statistically significant change in erythema scores, however, from day 15 to 28, erythema scores showed an estimated decrease of 0.021 (0.017-0.025) per day (Table 4-18).

In addition to age, Table 4-18 shows other possible risk factors of infant characteristics that may be associated with erythema in this population. The results from the bivariate analyses indicate that both birthweight and average number of massages per week during the first week of life were statistically significantly associated with erythema. For each 100g increase in birthweight, infants' erythema scores in this region increased an estimated 0.0084 (95% CI: 0.0017-0.015). For each additional massage received during the first week of life, infants' erythema scores in the chest region decreased 0.023 (95% CI: 0.00035-0.046).

Infants in this population tended to have increasing (worsening) rash scores in the chest region until day 14, with decreasing rash scores from day 14 to 28 (Figure 4-5). At visit 1, infants started with an estimated mean rash score of 0.15 (±0.42), reached a high rash score of 1.01 (±0.80) at visit 14, before having a lower mean rash score at visit 28 of 0.68 (±0.72) (Table 4-5). Between days 0 and 14, rash scores are estimated to increase 0.058 (95% CI: 0.052-0.063) per day. After day 14, rash scores are estimated to decrease 0.031 (95% CI: 0.025-0.036) per day (Table 4-19). Other characteristics that were statistically significantly associated with rash in the chest region in the bivariate models included: gestational age, birthweight, whether an infant was small-for-gestational age, average number of massages an infant received during the first week of life, and time from visit to last massage. Smaller infants (those who were preterm or of low birthweight) tended to have lower rash scores. Preterm infants had rash scores 0.083 (95% CI: 0.015-0.15) lower than full term infants. Infants who were between 32 and 36 weeks gestational age had rash scores 0.077 (95% CI: 0.0058-0.15) lower than full term infants. For each increase in week of gestational age, infants' rash scores increased an estimated 0.014 (95% CI: 0.0041-0.023). Low birthweight infants had rash scores 0.16 (95% CI: 0.092-0.23) lower than infants weighing 2500g or more at the birth visit. For each increase in 100g of birthweight, infants' rash scores increased 0.024 (95% CI: 0.017-0.031). In addition, infants who were small-for-gestational age and in the 3rd percentile or lower had rash scores 0.087 (95% CI: 0.0046-0.17) lower compared to infants who were adequate-for-gestational age (AGA). For each increase in number of times massaged per week infants had a 0.042 (95% CI: 0.019-0.068) lower rash score.

Also, infants who were massaged more than 180 minutes before the follow-up visit had estimated rash scores 0.097 (95% CI: 0.015-0.18) higher than infants massaged <30 minutes before the follow-up visit.

Table 4-20 shows the results of bivariate analyses of variables associated with the mother and the household that may affect skin erythema. These results indicate that whether the household had electricity, maternal age, facility delivery, and skilled attendant at delivery were statistically significantly associated with erythema. Infants whose mothers were older than 18 years of age had higher erythema scores than those whose mothers were younger than 18 years of age. Infants whose mothers were between 18 and 24 years old were estimated to have 0.097 (95% CI: 0.0013-0.19) higher skin erythema scores compared with infants whose mothers were less than 18 years old. Infants whose mothers were between 25 and 29 years old were estimated to have 0.11 (95% CI: 0.0016-0.22) higher erythema scores in the chest region compared with infants whose mothers were less than 18 years. Infants whose household had electricity had erythema values 0.094 (95% CI: 0.029-0.16) lower compared with infants whose households did not have electricity. Facility deliveries were associated with an estimated erythema score 0.13 (95% CI: 0.058-0.20) lower compared with home deliveries and infants who had a skilled attendant at delivery had estimated skin erythema scores 0.11 (95% CI: 0.038-0.17) lower compared with infants with no skilled attendant at delivery.

The bivariate analyses results of variables associated with the mother and the household that may affect skin rash scores are shown in Table 4-21. Ethnicity, maternal age, gravidity, and parity all showed a statistically significant association with skin rash scores. *Madeshi* infants had rash scores 0.17 (95% CI: 0.038-0.30) lower compared with

Pahadi infants. Increases in maternal age and numbers of previous pregnancies and deliveries were all associated with increases in rash scores. Mothers who were 18-24 years old at the time of birth and mothers who were 25-29 years old at the time of birth had infants with rash scores 0.15 (95% CI: 0.050-0.26) and 0.18 (95% CI: 0.058-0.29) higher respectively compared with infants whose mothers were less than 18 years old. In addition, infants whose mothers were between 30 and 34 years old had rash scores 0.19 (95% CI: 0.027-0.36) higher compared with infants whose mothers were less than 18 years old. Infants whose mother had 1-2 or 3 or more previous pregnancies had rash scores 0.098 (95% CI: 0.016-0.18) and 0.14 (95% CI: 0.050-0.23) higher respectively compared with infants whose mother had no previous pregnancies. Likewise, infants whose mother had 1-2 or 3 or more previous deliveries had rash skin scores 0.090 (95% CI: 0.0085-0.17) and 0.15 (95% CI: 0.060-0.27) higher respectively compared with infants whose mother had no previous deliveries.

The results of bivariate analyses of variables related to the environment and their impact on skin erythema scores are shown in Table 4-22. These results indicate that both temperature and heat index were statistically significantly associated with erythema. For each 10°C increase in temperature, skin erythema scores were estimated to decrease 0.26 (95% CI: 0.19-0.32). Similarly, with each 10°C increase in heat index, erythema scores were estimated to decrease 0.12 (95% CI: 0.085-0.15). There was no statistically significant association with erythema scores and relative humidity.

The results of bivariate analyses of variables related to the environment and their impact on skin rash scores for the chest region are shown in Table 4-23. Both relative humidity and heat index were statistically significantly associated with rash scores. For each 10% increase in relative humidity, rash scores increased 0.045 (95% CI: 0.019-0.07). For each 10°C increase in heat index, rash scores increased 0.055 (95% CI: 0.02-0.09). Temperature was not statistically significantly associated with rash scores, however there was a trend of increasing rash scores as temperature increased.

Table 4-24 shows the multivariate model constructed from variables that were statistically significantly associated with skin erythema in the bivariate analyses and with variables that may not have been statistically significantly associated with erythema in the bivariate analyses but are believed to be associated with erythema in this population. The variables included in the multivariate model were: preterm status, sex, ethnicity, birthweight, average number of massages per day during the first week of life, whether the household had electricity, maternal age, facility delivery, temperature, relative humidity, and infant's age between days 0 and 3, 4 and 14, and 15 and 28 (using linear splines to account for nonlinearity). Whether there was a skilled birth attendant at delivery and heat index were not included due to collinearity with other variables. Unlike in the bivariate analyses, in the multivariate model, there was no statistically significant association between erythema and average number of massages during the first week of life. In addition, there was no statistically significant association between either an infant's term status or sex on erythema. After controlling for other variables in the model, *Madeshi* infants' erythema scores were estimated to be 0.14 (95% CI: 0.017-0.27) lower than *Pahadi* infants' erythema scores. In the multivariate model, for each 100g increase in birthweight infants had an increase in erythema scores of 0.0073 (95% CI: 0.0002-0.014). Infants whose mothers were between 18 and 24 years old had erythema scores 0.10 (95% CI: 0.0096-0.20) higher compared with infants whose mothers were less than 18 years old. Whether a household had electricity was associated with a 0.088 (95% CI: 0.025-0.15) lower skin erythema score and facility deliveries were associated with a 0.11 (95% CI: 0.039-0.18) decrease in erythema scores. When controlling for other variables in the model, for each 10°C increase in temperature, erythema scores were estimated to decrease 0.25 (95% CI: 0.18-0.31). In the multivariate model throughout an infant's first month of life, erythema scores increased an estimated 0.13 (95% CI: 0.11-0.16) per day between days 0 and 3, showed no statistically significant change between days 4 and 14, and decreased an estimated of 0.022 (95% CI: 0.017-0.027) per day between days 15 and 28.

The multivariate model constructed from variables that were statistically significantly associated with skin rash in the bivariate analyses and with variables that may not have been statistically significantly associated with rash in the bivariate analyses but are believed to be associated with rash in this population is shown in Table 4-25. Variables included in the model were: preterm status, sex, ethnicity, birthweight, average number of massages received per day during the first week of life, time between follow-up visit and last massage, maternal age, parity, temperature, relative humidity, and infant's age from days 0 to 14 and days 15 to 28 (using splines to account for nonlinearity). The multivariate model showed no statistically significant association between skin rash scores and preterm status, sex, average number of massages per day during the first week of life, time between follow-up visit and last massage, or maternal age. These results estimate that when controlling for other variables in the model, *Madeshi* infants had rash scores 0.17 (95% CI: 0.032-0.31) lower compared with *Pahadi* infants. Also, infants with birthweight less than 2500g had rash scores 0.12 (95% CI: 0.044-0.19) lower than infants who weighed at least 2500g. Mothers with 3 or more prior deliveries had infants with rash scores 0.13 (95% CI: 0.017-0.25) higher compared with infants whose mothers had no prior deliveries. Although in the bivariate model there was not a statistically significant association between rash scores and temperature, in the multivariate model, for each 10°C increase in temperature, infants' rash scores were estimated to increase 0.13 (95% CI: 0.059-0.21). In addition, for each 10% increase in relative humidity, infants' rash scores were estimated to increase 0.053 (95% CI: 0.028-0.079). After controlling for other variables in the model, when an infant was between 0 and 14 days old, rash scores were estimated to increase 0.059 (95% CI: 0.053-0.064) per day. An infant's rash score was estimated to decrease 0.030 (95% CI: 0.025-0.036) per day between days 15 and 28.

Risk factor associations with skin erythema and rash were also examined for the left arm and the right leg. These regions showed very similar risk factor associations as the chest region with a few slight differences (Appendix A). Erythema in the left arm region showed a statistically significant association with gestational age and SGA in the bivariate analyses with smaller infants having lower erythema scores, however these associations were not statistically significant in the multivariate analysis. In addition, the average number of massages per day during the first week of life was not statistically significantly associated with erythema in the bivariate analysis for the left arm, unlike for the chest. There remained a statistically significant association between rash and preterm status for the left arm region in the multivariate model, unlike with the chest region, with preterm infants having lower rash scores.

When comparing the differences in variables associated with erythema and rash in the right leg region to the chest region, SGA was statistically significantly associated with erythema and rash (with smaller infants having lower scores) in the right leg region in the bivariate model, which was not the case in the chest region. However, this was not statistically significant in the multivariate model. Also, average number of massages per day during the first week was not statistically significantly associated with erythema in the right leg region in the bivariate model, while it was in the chest region. In the multivariate model for the right leg region, ethnicity was not statistically significantly associated with erythema, however it was statistically significant in the multivariate model for rash. Also, preterm status and time between follow-up visit and last massage were statistically significantly associated with skin rash in the right leg region in the multivariate model, with preterm infants having lower estimated rash scores and infants massaged more than 30 minutes before the follow-up visit having lower rash scores, with statistically significant differences seen in infants massaged between 30 and 119 minutes before the follow-up visit.

Table 4-26 shows how select infant and environmental characteristics were associated with the skin integrity measurements in the multivariate models. Preterm status was not statically significantly associated with any of the skin integrity measures in the multivariate models. Low birthweight infants were statistically significantly associated with higher skin pH and lower erythema and rash scores. Female infants had lower TEWL scores than male infants. Increasing temperature was statistically significantly associated with a decrease in all of the skin integrity measures except rash, with which it was associated with increasing scores. Increasing relative humidity was statistically significantly associated with increasing TEWL, skin pH, and rash scores and decreasing protein concentration.

Discussion

This study examined some of the possible risk factors related to skin integrity measures of TEWL, skin pH, stratum corneum protein concentration, and skin condition in a population of neonates in rural Nepal. We found that in this population, TEWL measures increased over the entire neonatal period with the greatest rate of increase per day occurring from days 0 to 3. This is in contrast to other studies that found TEWL measures in full term infants to be lower than or equal to adults and to remain relatively stable during the first month of life, indicating a highly effective skin barrier. (Cunico, Maibach et al. 1977; Yosipovitch, Maayan-Metzger et al. 2000) TEWL measures in preterm infants have also been shown to decrease relatively quickly during the first few days and then level off during the first month of life. (Agren, Sjörs et al. 1998; Kalia, Nonato et al. 1998) These differences in results could be explained by the differences in study design between our study and previous studies, as previous studies were done at low relative humidity. In addition, in one study the reported TEWL was corrected to a relative humidity of 50% (Agren, Sjörs et al. 1998) and another study was done in very premature babies who were in incubators. (Kalia, Nonato et al. 1998) To our knowledge, this is the first time TEWL has been measured in this type of population and environment (i.e. a community setting with consistently high humidity), therefore an infant's skin may not be undergoing the changes one would normally expect when adapting from a warm, humid environment in utero, to a cool, dry extrauterine environment. Relative humidity was high in our study environment where the average relative humidity was 77.8% (±11.7). In addition, relative humidity was associated with increasing TEWL in this study. This relationship of increasing TEWL with increasing humidity is similar to that found in a study where infants were randomized to care in an environment with 50% relative humidity (RH) or 75% RH. TEWL decreased more slowly in the group randomized to 75% RH during the first month of life and after 28 days the group at 75% RH had higher TEWL values, indicating delayed epidermal barrier maturation. (Agren, Sjörs et al. 2006) Our findings support this study, suggesting that the use of increasing relative humidity as a strategy for neonatal care in preterm infants should consider the impact this might have on delaying skin barrier maturation. (Fluhr, Darlenski et al. 2010)

In addition, although many studies have shown a relationship between TEWL and gestational age, with preterm infants having higher values of TEWL, we did not find a relationship between TEWL and gestational age in this population. (Hammarlund and Sedin 1979; Harpin and Rutter 1983) However, the most pronounced differences in TEWL between full term and preterm infants in these studies were found in infants with gestational ages less than 32 weeks, of which there were fewer than 40 in our study population, which may have made an association unable to be detected. Interestingly, being female in this population was associated with having lower TEWL measures compared with being male, whereas other studies have found no differences in TEWL values between sexes. (Harpin and Rutter 1983; Yosipovitch, Maayan-Metzger et al. 2000) Another interesting finding was that higher levels of measures of socioeconomic status, such as education and literacy, were associated with higher values of TEWL in this population.

Infants in this study had decreasing skin pH values during the first month of life, with the greatest rate of decrease occurring during the first week, similar to findings of other studies. (Visscher, Chatterjee et al. 2000; Yosipovitch, Maayan-Metzger et al. 2000; Hoeger and Enzmann 2002) Being low birthweight was also associated with higher skin pH values, in contrast with other studies finding no association between birthweight and skin pH. (Green, Carol et al. 1968) In addition, temperature and humidity showed differing relationships with skin pH, with increasing temperature being associated with decreasing skin pH and increasing humidity being associated with increasing in skin pH. Humidity may have a more important influence on the sweat response in infants in this population, as sweat may be unable to evaporate at such high humidity levels. As such, this increase in skin pH with increasing humidity could be a result of measuring the pH of sweat on the skin, which has been shown to result in higher skin pH measures. (Herrmann and Mandol 1955; Parra and Paye 2003)

Throughout the first month of life, infants showed a trend of decreasing stratum corneum protein concentrations removed per day during the first week followed by a slight increase in protein concentrations per day throughout the rest of the neonatal period. This indicates that during the first week of life, the stratum corneum is undergoing maturation as decreasing protein concentrations removed using the D-squame discs indicates increased cohesion of the stratum corneum. (Berthaud and Boncheva 2011; Myer and Maibach 2013) The slight increase in protein concentration per day after the first week of life could indicate the stratum corneum is starting to regenerate, causing old skin cells to shed and the stratum corneum to be less cohesive. (Chiou and Blume-Peytavi 2004) Time between visit and last massage was also associated with changes in stratum corneum protein concentration, with infants with greater time since last massage tending to have higher levels of protein concentration removed. This could be due to the universal use of emollients for massage in infants in this population, which could prevent protein from being removed if it is collected sooner after the massage has taken place as the barrier of oil between the disc and the stratum corneum may prevent removal. In addition, increased measures of SES status were associated with a decrease in protein concentration, indicating that infants who are better off may have better barrier function than infants who are less well off. Increases in temperature and humidity were also associated with a decrease in protein concentration. This again could be due to an increase in sweat that may be present, with the sweat acting as a barrier between the D-squame disc and the skin, resulting in less protein being removed. (Foster, Hey et al. 1969)

Erythema and rash are measures of irritation of the skin. The relationship between skin condition score and infant's age in this population indicated a worsening in erythema during the first days of life (days 0-3), with a leveling off period of very little to no change from days 4 to 14, and a period of improvement in the last two weeks. Rash scores tended to become worse during the first 2 weeks and to improve during the second 2 weeks of life. Smaller infants, those who were preterm or of low birthweight, were associated with lower erythema and rash scores than larger infants. These are similar to findings from a study by Visscher et al., which found lower perineal irritation (erythema and rash) in premature infants. (Visscher, Taylor et al. 2013) However, these data examined differences in a diapered region of the skin, which may not be comparable to data in our study. Higher measures of SES status were associated with improved erythema scores, however *Madeshi* infants, who are generally of lower SES than *Pahadi* infants had lower erythema and rash scores than *Pahadi* infants. This difference may be related to different skin pigmentations. Interestingly, increases in temperature were associated with a decrease in erythema, however increases in temperature and humidity were both associated with increases in rash scores. This is most likely due to the presence of miliaria, a common rash in newborns caused by retention of sweat due to immaturity of eccrine structures. (Zuniga and Nguyen 2013)

Although this study had many strengths including its large sample size and oversampling of preterm infants, it also had some limitations. Due to the fact that measurements were made at homes in this rural community it was sometimes difficult to follow the recommended measurement protocols for the instruments. For example, the standard TEWL measurement protocol normally requires the skin to be bare and in a still position for up to 30 minutes prior to measuring so that it acclimatizes to the environment. (Aki Immonen, personal communication) We were not able to do this in our study, which may have resulted in higher measurements as infants usually had their skin covered before and between measurements. In addition, it is recommended that TEWL measurements be taken in an environment that is less than 50% relative humidity and between 20-22°C, which never occurred during our study period. (Rogeries and Group 2001) A similar temperature controlled environment is recommended for skin pH measures. (Parra and Paye 2003) However, as temperature and humidity were controlled for in our multivariate models, this should not have impacted the associations of the other possible risk factors in those models.

In addition, a mother's recalled date of last menstrual period (LMP) was used to determine gestational age (GA), which may have resulted in inaccurate estimates of GA in our study. A study done in a tertiary-care hospital in Bangladesh found that LMP underestimated GA by one day compared with estimation from ultrasound. (Rosenberg, Ahmed et al. 2009) Another study done at a hospital in Pakistan found that only 65% and 82% of estimated GA of reported LMP were within 7 and 14 days, respectively when compared to GA estimates using ultrasound. (Jehan, Zaidi et al. 2010) Despite the problems of the uncertainty of LMP date, in this community setting in rural Nepal, the low-cost and simple method of reported LMP is the best option for estimating GA. In addition, LMPs in our study were collected as soon as a pregnancy was identified (following-up women every 5 weeks) which would minimize the amount of recall bias.

This study examined only some of the possible risk factors on skin barrier properties in this population. As neonatal oil massage is a nearly universal practice, we were not able to determine whether massage itself, different types of massage (i.e. vigorous vs. gentle), or massage with emollient were associated with skin integrity measurements. (Mullany, Darmstadt et al. 2005) However, we were able to examine the associations between average number of massages per day during the first week of life and time since last massage, which showed no statistically significant associations in the multivariate models, apart from time since massage with stratum corneum protein concentrations. Further research should be conducted in this population investigating whether different massage practices are associated with skin integrity measurements. This type of study would be difficult to do in this community due to cultural behaviors; however, a smaller group of neonates in an urban area may be more willing to accept behavioral change interventions relating to massage. Another possibility is to enroll older infants past the age when it is considered a cultural necessity to massage the infants, although this would not allow the measurement of skin maturity. Relationships between the different skin integrity measures should also be examined.

Newborn skin is an infant's first defense in protection against many environmental factors. If emollient therapy with vegetable oil is to be explored as a possible way of improving health in newborns, it is important to understand the risk factors associated with differences in skin barrier integrity in the populations that routinely practice neonatal oil massage. These same risk factors could be applicable to similar populations in northern India, Pakistan, and northwestern Bangladesh, as well as the Terai region in southern Nepal, as they share cultural, social, and economic characteristics. A better understanding of the underlying mechanisms of how and why emollient therapy can improve neonatal health outcomes in low-resource settings is important, however, additional risk factors associated with these biological measures need to be explored in order to optimize any health benefits of the oils. Further research on possible effects of oil massage on skin integrity, immune function, nutritional status, and bacterial colonization, along with risk factors associated with these biological mechanisms should be considered.

Chapter 4 Tables and Figures

Table 4-1: Scheduling of Visits and Associated Data Items Collected

Measure	# of Infants	Visit 1	Visit 3	Visit 7	Visit 14	Visit 28
Skin Condition Score	1000	X	X	X	X	X
TEWL	1000	X	X	X	X	X
Skin pH	1000	X	X	X	X	X
Skin Discs to Measure Protein Concentration	1000	X		X	X	X

Table 4-2: Baseline Newborn Care Characteristics

Characteristic	N	(%)
Sex		
Male	329	51.6
Female	308	48.4
Gestational Age		
<32 wks	37	5.9
32-36 wks	230	36.8
37-41 wks	307	49.1
>=42 wks	51	8.2
Weight at initial visit		
<1500g	12	1.9
1500-2499g	219	34.8
>2500g	399	63.3
SGA Status		
AGA	363	57.6
SGA 3-10%	119	18.9
SGA 3%	148	23.5
Breastfed since birth		
No	96	15.1
Yes	541	84.9
Hours before breastfeeding initiation		
<1 hr	161	30.1
1-2 hrs	305	57
3-4 hrs	35	6.5
>=5 hrs	34	6.4
Infant received colostrum		
No	57	10.5
Yes	483	89.3
Time from visit to last massage		
<30 min	615	20.8
30-59 min	505	17.1
60-119 min	681	23.0
120-179 min	398	13.5
>180 min	759	25.7
	MEAN	**SD**
Gestational Age (N=625)	37.7	3.6
Birthweight (N=632)	2611.8	483.8
Avg # of massages per day during first week of life	4.5	1.4

Table 4-3: Baseline Maternal, Socioeconomic, and Household Characteristics

Characteristic	N	(%)
Ethnic Group		
Pahadi	44	7.2
Madeshi	571	92.8
Maternal literacy		
Not literate	446	71.5
Literate	178	28.5
Paternal literacy		
Not literate	294	47.1
Literate	330	52.9
Maternal Education		
None	448	71.7
1-5 yrs	58	9.3
6-10 yrs	92	14.7
>10 yrs	27	4.3
Paternal Education		
None	290	46.4
1-5 yrs	94	15
6-10 yrs	201	32.2
>10 yrs	40	6.4
Household Assets[§]		
0 or 1 asset(s)	44	7.2
2-5 assets	276	44.9
6-10 assets	280	45.5
>10 assets	15	2.4
Electricity		
None	252	41
Yes	363	59
Maternal Age		
<18 yrs	83	13.3
18-24 yrs	353	56.6
25-29 yrs	135	21.6
30-34 yrs	35	5.6
>=35 yrs	18	2.9
Gravidity		
None	167	26.7
1-2	289	46.2
3-4	155	24.8
>5	14	2.2

Table 4-3: Baseline Maternal, Socioeconomic, and Household Characteristics (continued)

Characteristic	N	(%)
Parity		
None	180	28.8
1-2	265	42.4
3-4	155	24.8
>5	25	4
Antenatal Care Visits		
No ANC	182	29.2
1-2 ANC visits	227	36.4
3-4 ANC visits	188	30.1
>=5 ANC visits	27	4.3
Delivery Location		
Home	446	71.5
Facility	178	28.5
Length of labor		
<5 hrs	293	47
5-14 hrs	259	41.6
15-19 hrs	27	4.3
20-24 hrs	24	3.9
>24 hrs	20	3.2
Complications during delivery		
No	509	81.8
Yes	113	18.2
Delivery Assistant		
Unskilled	421	67.6
Skilled	202	32.4

§: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 4-4: Baseline Environmental Characteristics

Characteristic	N	(%)
Relative humidity (%)		
<40%	27	0.9
40-59%	223	7.4
60-79%	1,204	40.1
>80%	1,552	51.6
	MEAN	**SD**
Temperature (°C) (N=3003)	28.7	4.2
Relative humidity (%) (N=2991)	77.8	11.7
Heat index (°C) (N=2991)	35.9	9.0

Table 4-5: Descriptive Characteristics of Skin Integrity Measures

Visit	Measure (Mean (SD))				
	TEWL ($g/m^2/hr$)	Skin pH	Protein Concentration ($\mu g/cm^2$)	Chest Erythema Score	Chest Rash Score
1	34.8 (22.9)	6.03 (0.52)	16.4 (7.9)	0.53 (0.67)	0.15 (0.42)
3	37.7 (24.4)	5.59 (0.49)	N/A	0.83 (0.66)	0.70 (0.77)
7	39.2 (24.0)	5.19 (0.54)	12.9 (6.4)	0.80 (0.64)	0.80 (0.81)
14	41.6 (24.3)	5.07 (0.55)	13.1 (6.3)	0.83 (0.64)	1.01 (0.80)
28	43.3 (23.2)	4.82 (0.57)	14.0 (6.1)	0.53 (0.58)	0.68 (0.72)

Table 4-6: TEWL and Infant Characteristics Bivariate Analyses

Predictor Variable	Transepidermal Water Loss ($g/m^2/hr$)			
	Coefficient	SE	p-value	95% CI
Female	-3.77	1.36	0.006	-6.44- -1.09
Preterm (<37 wks)	-2.04	1.39	0.141	-4.76-0.67
Gestational Age[a]				
32-36 wks	-2.03	1.45	0.164	-4.88-0.82
<32 wks	-1.73	2.96	0.558	-7.54-4.07
Gestational Age (cont.) (wks)	0.17	0.19	0.388	-0.21-0.54
Low birthweight (<2500g)	-0.29	1.42	0.837	-3.07-2.48
Birthweight (g) (cont.)	0.0006	0.0014	0.681	-0.0022-0.0034
SGA status[b]				
SGA 3-10%	-0.55	1.81	0.759	-4.10-2.99
SGA 3%	1.64	1.68	0.330	-1.65-4.92
Breastfed since birth	-1.24	1.91	0.518	-4.99 -2.51
Hours before breastfeeding initiation[c]				
1-2 hrs	0.049	1.65	0.976	-3.18-3.28
3-4 hrs	-1.68	3.13	0.592	-7.83-4.46
>=5 hrs	6.44	3.18	0.043	0.21-12.68
Infant received colostrum	0.94	2.40	0.697	-3.77-5.65
Avg # of massages per day during first week of life	0.034	5.02	0.945	-0.95-1.02
Time from visit to last massage[d]				
30-59 min	-0.18	1.25	0.884	-2.62-2.26
60-119 min	-0.52	1.19	0.664	-2.84-1.81
120-179 min	-1.20	1.37	0.380	-3.89-1.48
>180 min	1.01	1.18	0.394	-1.31-3.32
Infant's age days 0 to 3	1.71	0.43	<0.001	0.86-2.55
Infant's age days 4 to 28	0.22	0.04	<0.001	0.13- 0.30

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour

Table 4-7: TEWL and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Transepidermal Water Loss ($g/m^2/hr$)			
	Coefficient	SE	p-value	95% CI
Madeshi	-4.55	2.69	0.090	-9.82-0.72
Mother literate	3.40	1.52	0.025	0.43-6.37
Father literate	0.53	1.38	0.702	-2.17-3.22
Maternal Education[a]				
1-5 yrs	2.59	2.39	0.278	-2.09-7.28
6-10 yrs	3.08	1.96	0.117	-0.77-6.93
>10 yrs	0.28	3.39	0.935	-6.37-6.93
Paternal Education[a]				
1-5 yrs	0.50	2.03	0.807	-3.48-4.48
6-10 yrs	0.40	1.58	0.802	-2.69-3.48
>10 yrs	-0.66	2.91	0.820	-6.37-5.05
Household Assets[b, §]				
2-5 assets	-0.57	2.82	0.841	-6.11-4.97
6-10 assets	-0.22	2.83	0.939	-5.76-5.32
>10 assets	2.32	5.17	0.653	-7.81-12.46
Household has electricity	3.71	1.41	0.008	0.96-6.47
Maternal Age[c]				
18-24 yrs	0.86	2.10	0.681	-3.25-4.97
25-29 yrs	2.12	2.39	0.376	-2.57-6.81
30-34 yrs	0.58	3.45	0.866	-6.17-7.33
>=35 yrs	4.20	4.45	0.345	-4.52-12.93
Gravidity[d]				
1-2	-0.09	1.67	0.956	-3.36-3.18
>=3	-0.58	1.87	0.758	-4.24-3.09
Parity[d]				
1-2	-0.03	1.66	0.986	-3.28-3.22
>=3	-0.76	1.81	0.674	-4.31-2.78
Antenatal Care Visits[d]				
1-2 ANC visits	5.49	1.69	0.001	2.18-8.80
>=3 ANC visits	5.28	1.71	0.002	1.93-8.64
Delivery at facility	-1.18	1.43	0.438	-4.15-1.80
Length of labor[e]				
5-14 hrs	-2.32	1.46	0.113	-5.18-0.54
>=15 hrs	1.08	2.27	0.632	-3.36-5.53
Complications during delivery	-0.39	1.77	0.824	-3.88-3.09
Skilled assistant at delivery	-1.70	1.47	0.248	-4.57-1.18

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 4-8: TEWL and Environmental Characteristics Bivariate Analyses

Predictor Variable	Transepidermal Water Loss ($g/m^2/hr$)			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	-1.62	0.12	<0.001	-1.86- -1.38
Relative humidity (cont.) (%)	0.51	0.039	<0.001	0.43-0.59
Relative humidity (%)[a]				
40-59%	-4.03	4.13	0.328	-12.12-4.05
60-79%	6.86	3.91	0.080	-0.81-14.53
>80%	13.95	3.91	<0.001	6.28-21.62
Heat index (°C)	-0.49	0.061	<0.001	-0.61- -0.37

a: Reference group, <40%

Table 4-9: TEWL Multivariate Model

Predictor Variable	Transepidermal Water Loss ($g/m^2/hr$)			
	Coefficient	SE	p-value	95% CI
Preterm (<37 wks)	1.45	1.36	0.296	-1.21-4.11
Female	-2.99	1.32	0.024	-5.57- -0.40
Madeshi	-4.38	2.47	0.076	-9.21-0.46
Mother literate	2.66	1.53	0.083	-0.34-5.66
Household has electricity	1.70	1.41	0.228	-1.06-4.45
Antenatal Care Visits[a]				
1-2 ANC visits	4.68	1.66	0.005	1.42-7.94
>=3 ANC visits	3.87	1.71	0.023	0.53-7.22
Hours before breastfeeding initiation[b]				
1-2 hrs	0.39	1.50	0.795	-2.54-3.32
3-4 hrs	-1.54	2.83	0.588	-7.09-4.02
>=5 hrs	5.89	2.86	0.039	0.29-11.49
Temperature (°C)	-1.24	0.14	<0.001	-1.50- -0.97
Relative humidity (%)	0.36	0.04	<0.001	0.28-0.45
Infant's age days 0 to 3	0.96	0.46	0.038	0.05-1.87
Infant's age days 4 to 28	0.20	0.05	<0.001	0.11-0.29

a: Reference group, none; b: Reference group, <1 hour

Table 4-10: Skin pH and Infant Characteristics Bivariate Analyses

Predictor Variable	Skin pH			
	Coefficient	SE	p-value	95% CI
Female	0.017	0.029	0.552	-0.039-0.074
Preterm (<37 wks)	0.010	0.029	0.724	-0.047-0.068
Gestational Age[a]				
32-36 wks	0.0021	0.031	0.946	-0.058-0.062
<32 wks	0.087	0.063	0.165	-0.036-0.21
Gestational Age (cont.) (wks)	-0.0056	0.0041	0.170	-0.013-0.0024
Low birthweight (<2500g)	0.089	0.030	0.002	0.032-0.15
Birthweight (g) (cont.)	-0.00011	0.000030	<0.001	-0.00017- -0.000050
SGA status[b]				
SGA 3-10%	0.026	0.038	0.495	-0.049-0.10
SGA 3%	0.055	0.036	0.119	-0.014-0.13
Breastfed since birth	-0.076	0.040	0.060	-0.15-0.0030
Hours before breastfeeding initiation[c]				
1-2 hrs	0.017	0.036	0.640	-0.053-0.086
3-4 hrs	0.031	0.067	0.647	-0.10-0.16
>=5 hrs	0.087	0.068	0.203	-0.047-0.22
Infant received colostrum	0.036	0.051	0.483	-0.065-0.14
Avg # of massages per day during first week of life	0.011	0.010	0.296	-0.0096-0.031
Time from visit to last massage[d]				
30-59 min	-0.0091	0.040	0.822	-0.088-0.070
60-119 min	-0.073	0.038	0.055	-0.15-0.0016
120-179 min	-0.089	0.044	0.041	-0.18- -0.0037
>180 min	-0.23	0.037	<0.001	-0.30- -0.16
Infant's age days 0 to 7	-0.14	0.004	<0.001	-0.14- -0.13
Infant's age days 8 to 28	-0.016	0.001	<0.001	-0.018- -0.014

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 minutes

Table 4-11: Skin pH and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Skin pH			
	Coefficient	SE	p-value	95% CI
Madeshi	0.15	0.057	0.007	0.043-0.26
Mother literate	-0.080	0.032	0.013	-0.14- -0.017
Father literate	-0.082	0.029	0.005	-0.14- -0.025
Maternal Education[a]				
1-5 yrs	-0.10	0.050	0.047	-0.20- -0.0011
6-10 yrs	-0.063	0.041	0.126	-0.14-0.018
>10 yrs	-0.058	0.072	0.423	-0.20-0.084
Paternal Education[a]				
1-5 yrs	-0.027	0.042	0.522	-0.11-0.056
6-10 yrs	-0.11	0.033	0.001	-0.17- -0.045
>10 yrs	-0.060	0.062	0.331	-0.18-0.061
Household Assets[b, §]				
2-5 assets	-0.11	0.059	0.073	-0.22-0.0098
6-10 assets	-0.14	0.059	0.016	-0.26- -0.026
>10 assets	-0.28	0.11	0.010	-0.49- -0.066
Household has electricity	-0.077	0.030	0.010	-0.13- -0.019
Maternal Age[c]				
18-24 yrs	-0.077	0.044	0.080	-0.16-0.0092
25-29 yrs	-0.061	0.050	0.224	-0.16-0.037
30-34 yrs	-0.097	0.072	0.179	-0.24-0.044
>=35 yrs	-0.086	0.093	0.355	-0.27-0.096
Gravidity[d]				
1-2	-0.028	0.035	0.422	-0.098-0.041
>=3	0.0011	0.039	0.977	-0.076-0.078
Parity[d]				
1-2	-0.023	0.035	0.503	-0.092-0.045
>=3	0.0056	0.038	0.884	-0.069-0.080
Antenatal Care Visits[d]				
1-2 ANC visits	-0.010	0.036	0.779	-0.081-0.061
>=3 ANC visits	-0.0016	0.037	0.966	-0.073-0.070
Delivery at facility	-0.076	0.032	0.018	-0.14- -0.013
Length of labor[e]				
5-14 hrs	0.012	0.031	0.693	-0.048-0.073
>=15 hrs	0.051	0.048	0.286	-0.043-0.14
Complications during delivery	-0.021	0.38	0.572	-0.095-0.052
Skilled assistant at delivery	-0.085	0.031	0.006	-0.15- -0.025

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 4-12: Skin pH and Environmental Characteristics Bivariate Analyses

Predictor Variable	Skin pH			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	-0.019	0.0032	<0.001	-0.025- -0.013
Relative humidity (cont.) (%)	0.0078	0.0011	<0.001	0.0056-0.010
Relative humidity (%)[a]				
40-59%	-0.35	0.14	0.011	-0.61- -0.077
60-79%	-0.36	0.13	0.006	-0.61- -0.10
>80%	-0.17	0.13	0.198	-0.42- 0.087
Heat index (°C)	-0.0050	0.0015	0.001	-0.0080- -0.0020

a: Reference group, <40%

Table 4-13: Skin pH Multivariate Model

Predictor Variable	Skin pH			
	Coefficient	SE	p-value	95% CI
Preterm (<37 wks)	-0.0072	0.028	0.800	-0.063-0.049
Female	0.037	0.028	0.183	-0.018-0.092
Madeshi	0.13	0.056	0.016	0.025-0.25
Low birthweight (<2500g)	0.059	0.029	0.044	0.0015-0.12
Mother literate	-0.040	0.035	0.252	-0.11-0.028
Father literate	-0.040	0.033	0.226	-0.10-0.025
Household has electricity	-0.043	0.031	0.169	-0.10-0.018
Household Assets[a, §]				
2-5 assets	-0.07	0.056	0.220	-0.18-0.041
6-10 assets	-0.062	0.061	0.311	-0.18-0.058
>10 assets	-0.14	0.11	0.201	-0.35-0.074
Skilled Attendant at delivery	-0.013	0.031	0.658	-0.074-0.047
Time between follow-up visit and last massage[b]				
30-59 min	0.015	0.029	0.596	-0.041-0.072
60-119 min	-0.028	0.028	0.308	-0.082-0.026
120-179 min	-0.0040	0.032	0.898	-0.066-0.058
>180 min	-0.050	0.028	0.067	-0.104-0.0035
Temperature (°C)	-0.023	0.0029	<0.001	-0.029- -0.017
Relative humidity (%)	0.0057	0.00094	<0.001	0.0038-0.0075
Infant's age days 0 to 7	-0.14	0.0038	<0.001	-0.15- -0.13
Infant's age days 8 to 28	-0.015	0.0013	<0.001	-0.017- -0.012

a: Reference group, 0 or 1 asset(s); b: Reference group, <30 min; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 4-14: Stratum Corneum Protein Concentration and Infant Characteristics Bivariate Analyses

Predictor Variable	Protein Concentration (μg/cm²)			
	Coefficient	SE	p-value	95% CI
Female	0.39	0.35	0.266	-0.30-1.08
Preterm (<37 wks)	0.48	0.36	0.180	-0.22-1.17
Gestational Age[a]				
32-36 wks	0.44	0.37	0.241	-0.29-1.16
<32 wks	0.96	0.76	0.205	-0.53-2.45
Gestational Age (cont.) (wks)	-0.088	0.049	0.072	-0.18-0.0079
Low birthweight (<2500g)	0.57	0.36	0.112	-0.13-1.28
Birthweight (g) (cont.)	-0.00036	0.00037	0.326	-0.0011-0.00037
SGA status[b]				
SGA 3-10%	-0.49	0.46	0.291	-1.40-0.42
SGA 3%	-0.37	0.43	0.387	-1.22-0.47
Breastfed since birth	-0.29	0.48	0.551	-1.24-0.66
Hours before breastfeeding initiation[c]				
1-2 hrs	0.20	0.44	0.644	-0.66-1.07
3-4 hrs	0.60	0.83	0.470	-1.02-2.22
>=5 hrs	0.69	0.82	0.399	-0.91-2.30
Infant received colostrum	-0.89	0.65	0.169	-2.16-0.38
Avg # of massages per day during first week of life	-0.12	0.13	0.352	-0.37-0.13
Time from visit to last massage[d]				
30-59 min	0.25	0.51	0.628	-0.75-1.25
60-119 min	1.47	0.47	0.002	0.54-2.40
120-179 min	1.22	0.55	0.026	0.15-2.30
>180 min	1.78	0.46	<0.001	0.88-2.68
Infant's age days 0 to 7	-0.58	0.06	<0.001	-0.70- -0.46
Infant's age days 8 to 28	0.06	0.02	0.003	0.02-0.10

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 min

Table 4-15: Stratum Corneum Protein Concentration and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Protein Concentration (μg/cm^2)			
	Coefficient	SE	p-value	95% CI
Madeshi	-0.67	0.72	0.351	-2.09-0.74
Mother literate	-0.37	0.39	0.343	-1.14-0.40
Father literate	-0.92	0.35	0.009	-1.60- -0.23
Maternal Education[a]				
1-5 yrs	-0.69	0.60	0.248	-1.86-0.48
6-10 yrs	-0.35	0.51	0.493	-1.35-0.65
>10 yrs	1.12	0.91	0.220	-0.67-2.91
Paternal Education[a]				
1-5 yrs	-0.04	0.51	0.937	-1.04-0.96
6-10 yrs	-1.08	0.40	0.007	-1.87- -0.29
>10 yrs	-1.27	0.78	0.102	-2.79-0.25
Household Assets[b, §]				
2-5 assets	-0.19	0.71	0.793	-1.16-0.88
6-10 assets	-0.66	0.71	0.351	-2.05-0.73
>10 assets	0.43	1.27	0.733	-2.05-2.92
Household has electricity	-0.85	0.36	0.017	-1.55- -0.15
Maternal Age[c]				
18-24 yrs	-0.80	0.54	0.141	-1.87-0.27
25-29 yrs	-1.15	0.62	0.063	-2.36-0.063
30-34 yrs	-0.04	0.87	0.960	-1.75-1.66
>=35 yrs	0.04	1.05	0.973	-2.03-2.10
Gravidity[d]				
1-2	-0.14	0.43	0.751	-0.98-0.71
>=3	0.23	0.48	0.635	-0.71-1.16
Parity[d]				
1-2	-0.23	0.43	0.598	-1.06-0.61
>=3	0.34	0.46	0.456	-0.56-1.25
Antenatal Care Visits[d]				
1-2 ANC visits	-1.07	0.44	0.014	-1.93- -0.22
>=3 ANC visits	-0.83	0.45	0.064	-1.71-0.048
Delivery at facility	-0.54	0.39	0.170	-1.32-0.23
Length of labor[e]				
5-14 hrs	-0.89	0.37	0.017	-1.62- -0.16
>=15 hrs	-0.43	0.58	0.541	-1.57-0.70

Table 4-15: Stratum Corneum Protein Concentration and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses (continued)

Predictor Variable	Protein Concentration (µg/cm^2)			
	Coefficient	SE	p-value	95% CI
Complications during delivery	0.48	0.47	0.301	-0.43-1.40
Skilled assistant at delivery	-0.69	0.38	0.070	-1.44-0.06

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 4-16: Stratum Corneum Protein Concentration and Environmental Characteristics Bivariate Analyses

Predictor Variable	Protein Concentration (µg/cm^2)			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	-0.20	0.04	<0.001	-0.27- -0.13
Relative humidity (cont.) (%)	-0.06	0.01	<0.001	-0.09- -0.04
Relative humidity (%)[a]				
40-59%	-0.18	1.60	0.910	-3.32-2.96
60-79%	-1.51	1.53	0.325	-4.51-1.49
>80%	-2.71	1.53	0.077	-5.72-0.29
Heat index (°C)	-0.14	0.02	<0.001	-0.17- -0.10

a: Reference group, <40%

Table 4-17: Stratum Corneum Protein Concentration Multivariate Model

Predictor Variable	Protein Concentration (μg/cm^2)			
	Coefficient	SE	p-value	95% CI
Preterm (<37 wks)	0.54	0.33	0.103	-0.11-1.18
Female	0.55	0.33	0.089	-0.084-1.19
Madeshi	-0.092	0.68	0.893	-1.42-1.24
Father literate	-0.96	0.35	0.005	-1.64- -0.28
Household has electricity	-0.62	0.35	0.075	-1.30-0.062
Antenatal Care Visits[a]				
1-2 ANC visits	-0.67	0.41	0.099	-1.46-0.12
>=3 ANC visits	-0.19	0.42	0.661	-1.02-0.64
Length of Labor				
5-14 hrs	-0.79	0.34	0.022	-1.46- -0.12
>=15 hrs	-0.55	0.53	0.302	-1.60-0.50
Time from visit to last massage[b]				
30-59 min	0.29	0.49	0.561	-0.68-1.25
60-119 min	1.55	0.46	0.001	0.65-2.44
120-179 min	1.43	0.53	0.007	0.39-2.48
>180 min	1.34	0.45	<0.001	1.46-3.22
Temperature (°C)	-0.31	0.037	<0.001	-0.38- -0.24
Relative humidity (%)	-0.084	0.013	<0.001	-0.11- -0.058
Infant's age days 0 to 7	-0.57	0.062	<0.001	-0.69- -0.45
Infant's age days 8 to 28	0.045	0.020	0.028	0.0047-0.085

a: Reference group, none; b: Reference group, <30 min

Table 4-18: Chest Erythema Score and Infant Characteristics Bivariate Analyses

Predictor Variable	Chest Erythema Score			
	Coefficient	SE	p-value	95% CI
Female	0.011	0.032	0.731	-0.052-0.074
Preterm (<37 wks)	0.011	0.033	0.738	-0.053-0.075
Gestational Age[a]				
32-36 wks	0.023	0.034	0.498	-0.044-0.090
<32 wks	-0.052	0.070	0.460	-0.19-0.085
Gestational Age (cont.) (wks)	-0.0013	0.0045	0.767	-0.010-0.0075
Low birthweight (<2500g)	-0.019	0.033	0.560	-0.084- 0.046
Birthweight (g) (cont.)	0.000084	0.000034	0.014	0.000017-0.00015
SGA status[b]				
SGA 3-10%	0.025	0.043	0.551	-0.058-0.11
SGA 3%	-0.038	0.040	0.341	-0.12-0.040
Breastfed since birth	-0.076	0.045	0.090	-0.16-0.012
Hours before breastfeeding initiation[c]				
1-2 hrs	-0.0012	0.039	0.976	-0.078-0.075
3-4 hrs	-0.037	0.074	0.615	-0.18-0.11
>=5 hrs	0.078	0.075	0.301	-0.069-0.22
Infant received colostrum	0.047	0.057	0.410	-0.065-0.16
Avg # of massages per day during first week of life	-0.023	0.012	0.047	-0.046- -0.00035
Time from visit to last massage[d]				
30-59 min	-0.041	0.037	0.268	-0.11-0.032
60-119 min	-0.063	0.035	0.073	-0.13-0.0060
120-179 min	-0.032	0.041	0.429	-0.11-0.048
>180 min	-0.026	0.035	0.460	-0.094-0.043
Infant's age days 0 to 3	0.14	0.014	<0.001	0.11-0.17
Infant's age days 4 to 14	0.00037	0.003	0.904	-0.0056-0.0063
Infant's age days 15 to 28	-0.021	0.002	<0.001	-0.025- -0.017

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 minutes

Table 4-19: Chest Rash Score and Infant Characteristics Bivariate Analyses

Predictor Variable	Chest Rash Score			
	Coefficient	SE	p-value	95% CI
Female	0.0043	0.034	0.902	-0.063-0.072
Preterm (<37 wks)	-0.083	0.035	0.017	-0.15- -0.015
Gestational Age[a]				
32-36 wks	-0.077	0.036	0.034	-0.15- -0.0058
<32 wks	-0.13	0.075	0.088	-0.27-0.019
Gestational Age (cont.) (wks)	0.014	0.0047	0.005	0.0041-0.023
Low birthweight (<2500g)	-0.16	0.035	<0.001	-0.23- -0.092
Birthweight (g) (cont.)	0.00024	0.000035	<0.001	0.00017-0.00031
SGA status[b]				
SGA 3-10%	0.041	0.045	0.364	-0.048-0.13
SGA 3%	-0.087	0.042	0.039	-0.17- -0.0046
Breastfed since birth	-0.018	0.048	0.704	-0.11-0.076
Hours before breastfeeding initiation[c]				
1-2 hrs	-0.00060	0.043	0.989	-0.084-0.085
3-4 hrs	-0.11	0.081	0.165	-0.27-0.046
>=5 hrs	-0.010	0.082	0.903	-0.17-0.15
Infant received colostrum	0.074	0.062	0.234	-0.048-0.20
Avg # of massages per day during first week of life	-0.042	0.012	0.001	-0.068- -0.019
Time from visit to last massage[d]				
30-59 min	0.024	0.045	0.591	-0.065-0.11
60-119 min	-0.0082	0.043	0.848	-0.092-0.075
120-179 min	0.041	0.049	0.406	-0.056-0.14
>180 min	0.097	0.042	0.021	0.015-0.18
Infant's age days 0 to 14	0.058	0.003	<0.001	0.052-0.063
Infant's age days 15 to 28	-0.031	0.003	<0.001	-0.036- -0.025

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 minutes

Table 4-20: Chest Erythema Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Chest Erythema Score			
	Coefficient	SE	p-value	95% CI
Madeshi	-0.12	0.063	0.055	-0.24-0.0023
Mother literate	-0.027	0.036	0.444	-0.097-0.043
Father literate	0.014	0.032	0.671	-0.050-0.077
Maternal Education[a]				
1-5 yrs	-0.00091	0.056	0.987	-0.11-0.11
6-10 yrs	-0.049	0.046	0.291	-0.14-0.042
>10 yrs	-0.0015	0.080	0.985	-0.16-0.16
Paternal Education[a]				
1-5 yrs	0.016	0.048	0.735	-0.077-0.11
6-10 yrs	0.010	0.037	0.784	-0.062-0.083
>10 yrs	-0.054	0.069	0.432	-0.19-0.081
Household Assets[b, §]				
2-5 assets	-0.032	0.066	0.630	-0.16-0.097
6-10 assets	-0.059	0.066	0.366	-0.19-0.069
>10 assets	-0.11	0.12	0.363	-0.35-0.13
Household has electricity	-0.094	0.033	0.004	-0.16- -0.029
Maternal Age[c]				
18-24 yrs	0.097	0.049	0.047	0.0013-0.19
25-29 yrs	0.11	0.056	0.047	0.0016-0.22
30-34 yrs	0.010	0.080	0.214	-0.058-0.26
>=35 yrs	0.075	0.10	0.467	-0.13-0.28
Gravidity[d]				
1-2	0.064	0.039	0.102	-0.013-0.14
>=3	0.053	0.044	0.230	-0.033-0.14
Parity[d]				
1-2	0.052	0.039	0.179	-0.024-0.13
>=3	0.044	0.042	0.296	-0.039-0.13
Antenatal Care Visits[d]				
1-2 ANC visits	0.045	0.043	0.263	-0.034-0.12
>=3 ANC visits	0.034	0.045	0.404	-0.046-0.11
Delivery at facility	-0.13	0.035	<0.001	-0.20- -0.058
Length of labor[e]				
5-14 hrs	-0.0021	0.034	0.952	-0.069-0.065
>=15 hrs	-0.016	0.053	0.766	-0.12-0.089

Table 4-20: Chest Erythema Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses (continued)

Predictor Variable	Chest Erythema Score			
	Coefficient	SE	p-value	95% CI
Complications during delivery	-0.058	0.042	0.166	-0.14-0.024
Skilled assistant at delivery	-0.11	0.034	0.002	-0.17- -0.038

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 4-21: Chest Rash Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Chest Rash Score			
	Coefficient	SE	p-value	95% CI
Madeshi	-0.17	0.067	0.011	-0.30- -0.038
Mother literate	0.0022	0.038	0.953	-0.073-0.077
Father literate	0.051	0.035	0.138	-0.016-0.12
Maternal Education[a]				
1-5 yrs	-0.016	0.060	0.793	-0.13-0.10
6-10 yrs	-0.022	0.049	0.649	-0.12-0.074
>10 yrs	0.011	0.086	0.218	-0.063-0.28
Paternal Education[a]				
1-5 yrs	0.012	0.051	0.813	-0.088-0.11
6-10 yrs	0.049	0.040	0.215	-0.028-0.13
>10 yrs	0.057	0.074	0.442	-0.088-0.20
Household Assets[b, §]				
2-5 assets	0.013	0.070	0.858	-0.13-0.15
6-10 assets	0.006	0.070	0.930	-0.13-0.14
>10 assets	0.11	0.13	0.399	-0.14-0.36
Household has electricity	-0.040	0.035	0.258	-0.11-0.029
Maternal Age[c]				
18-24 yrs	0.15	0.052	0.004	0.050-0.26
25-29 yrs	0.18	0.060	0.003	0.058-0.29
30-34 yrs	0.19	0.086	0.023	0.027-0.36
>=35 yrs	0.19	0.11	0.084	-0.025-0.41
Gravidity[d]				
1-2	0.098	0.042	0.020	0.016- 0.18
>=3	0.14	0.047	0.003	0.050-0.23
Parity[d]				
1-2	0.090	0.041	0.030	0.0085-0.17
>=3	0.15	0.045	0.001	0.060-0.27

Table 4-21: Chest Rash Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses (continued)

Predictor Variable	Chest Rash Score			
	Coefficient	SE	p-value	95% CI
Antenatal Care Visits[d]				
1-2 ANC visits	0.049	0.043	0.255	-0.035-0.13
>=3 ANC visits	0.027	0.043	0.542	-0.059-0.11
Delivery at facility	-0.028	0.038	0.468	-0.10-0.047
Length of labor[e]				
5-14 hrs	0.027	0.037	0.471	-0.046-0.099
>=15 hrs	-0.087	0.057	0.126	-0.20-0.024
Complications during delivery	-0.013	0.045	0.775	-0.10-0.075
Skilled assistant at delivery	-0.029	0.037	0.428	-0.10-0.043

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 4-22: Chest Erythema Score and Environmental Characteristics Bivariate Analyses

Predictor Variable	Chest Erythema Score			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	-0.026	0.0033	<0.001	-0.032- -0.019
Relative humidity (cont.) (%)	0.0022	0.0011	0.053	-0.000029-0.0045
Relative humidity (%)[a]				
40-59%	0.080	0.13	0.526	-0.17-0.33
60-79%	0.13	0.12	0.284	-0.11-0.37
>80%	0.12	0.12	0.330	-0.12-0.35
Heat index (°C)	-0.012	0.0016	<0.001	-0.015- -0.0085

a: Reference group, <40%

Table 4-23: Chest Rash and Environmental Characteristics Bivariate Analyses

Predictor Variable	Chest Rash			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	0.0065	0.0038	0.085	-0.0009-0.014
Relative humidity (cont.) (%)	0.0045	0.0013	0.001	0.0019-0.007
Relative humidity (%)[a]				
40-59%	-0.00091	0.15	0.995	-0.30-0.30
60-79%	0.13	0.12	0.361	-0.15-0.42
>80%	0.12	0.12	0.259	-0.12-0.45
Heat index (°C)	0.0055	0.0018	0.002	0.0020-0.0090

a: Reference group, <40%

Table 4-24: Chest Erythema Score Multivariate Model

Predictor Variable	Chest Erythema Score			
	Coefficient	SE	p-value	95% CI
Preterm (<37 wks)	0.025	0.032	0.452	-0.039-0.088
Female	0.027	0.032	0.400	-0.036-0.089
Madeshi	-0.14	0.065	0.026	-0.27- -0.017
Birthweight (g) (cont.)	0.000073	0.000036	0.044	0.000002-0.00014
Avg # of massages per day during first week of life	-0.0024	0.012	0.843	-0.026-0.021
Household has electricity	-0.088	0.032	0.006	-0.15- -0.025
Maternal Age[a]				
18-24 yrs	0.10	0.048	0.031	0.0096-0.20
25-29 yrs	0.082	0.056	0.143	-0.028-0.19
30-34 yrs	0.062	0.078	0.430	-0.091-0.22
>=35 yrs	0.026	0.099	0.797	-0.17-0.22
Facility delivery	-0.11	0.036	0.002	-0.18- -0.039
Temperature (°C)	-0.025	0.0034	<0.001	-0.031- -0.018
Relative humidity (%)	0.0010	0.0011	0.373	-0.0033-0.0012
Infant's age days 0 to 3	0.13	0.014	<0.001	0.11-0.16
Infant's age days 4 to 14	0.0017	0.0031	0.592	-0.0044-0.0078
Infant's age days 15 to 28	-0.022	0.0024	<0.001	-0.027- -0.017

a: Reference group, <18 years

Table 4-25: Chest Rash Score Multivariate Model

Predictor Variable	Chest Rash Score			
	Coefficient	SE	p-value	95% CI
Preterm (<37 wks)	-0.063	0.035	0.068	-0.13-0.0046
Female	0.013	0.034	0.688	-0.053-0.080
Madeshi	-0.17	0.072	0.016	-0.31- -0.032
Low birthweight (<2500 g)	-0.12	0.036	0.002	-0.19- -0.044
Avg # of massages per day during first week of life	-0.016	0.013	0.232	-0.045-0.0065
Time from visit to last massage[a]				
30-59 min	0.0071	0.043	0.869	-0.078-0.092
60-119 min	-0.044	0.041	0.288	-0.12-0.037
120-179 min	-0.025	0.047	0.602	-0.12-0.068
>180 min	-0.012	0.041	0.768	-0.093-0.069
Maternal Age[b]				
18-24 yrs	0.065	0.057	0.255	-0.047-0.18
25-29 yrs	0.033	0.074	0.660	-0.11-0.18
30-34 yrs	0.032	0.095	0.739	-0.16-0.22
>=35 yrs	0.043	0.119	0.719	-0.19-0.28
Parity[c]				
1-2	0.050	0.046	0.276	-0.040-0.14
>=3	0.13	0.060	0.025	0.017-0.25
Temperature (°C)	0.013	0.0038	<0.001	0.0059-0 .021
Relative humidity (%)	0.0053	0.0013	<0.001	0.0028-0.0079
Infant's age days 0 to 14	0.059	0.0029	<0.001	0.053-0.064
Infant's age days 15 to 28	-0.030	0.0029	<0.001	-0.036- -0.025

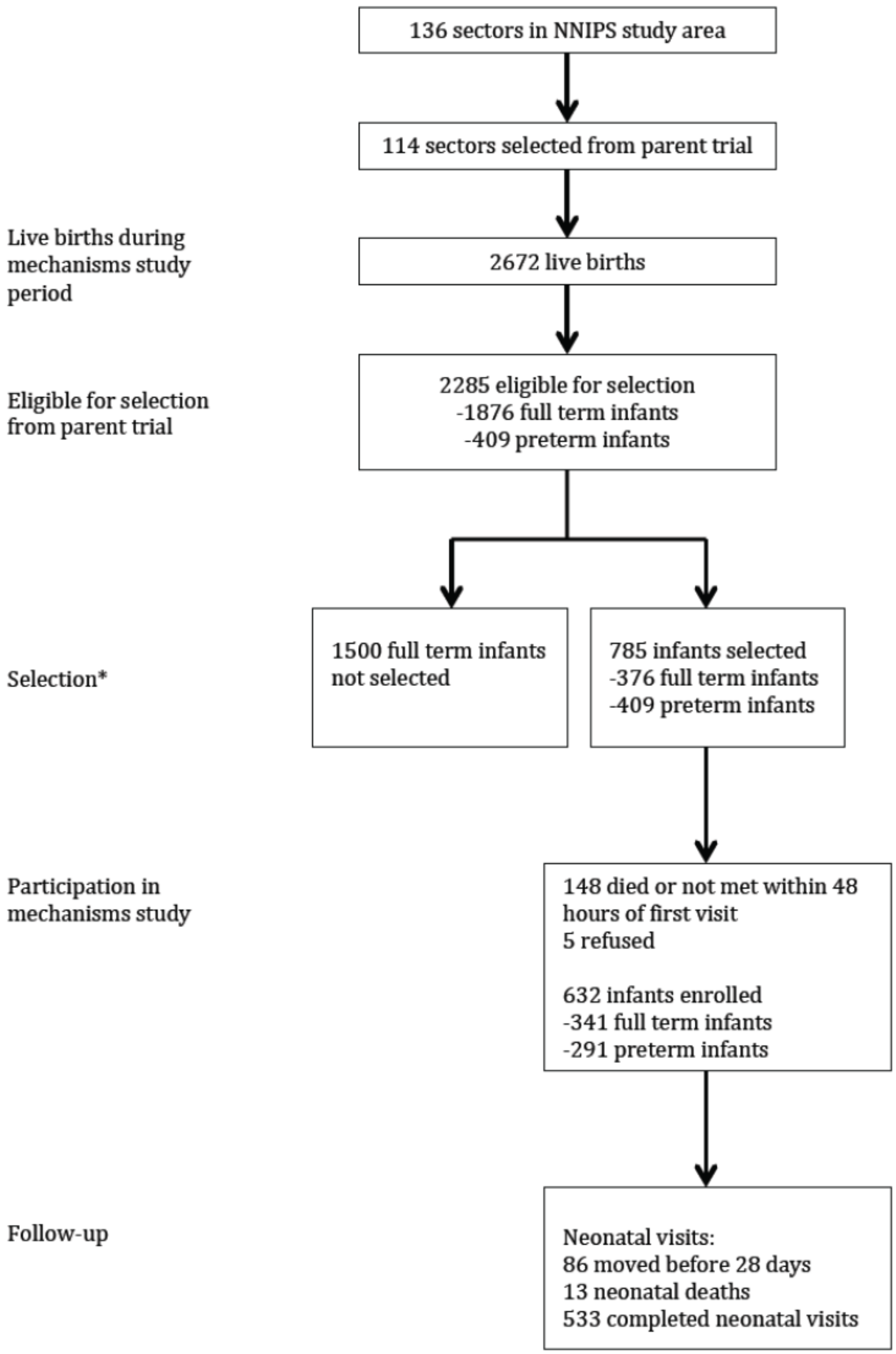
a: Reference group, <30 min; b: Reference group, <18 years; c: Reference group, none

Table 4-26: Direction of Association of Select Infant and Environmental Characteristics on Skin Integrity Measures in Multivariate Models

	Measurement				
	TEWL ($g/m^2/hr$)	Skin pH	Protein Concentration ($\mu g/cm^2$)	Chest Erythema Scores	Chest Rash Scores
Infant Characteristics					
Preterm (<37 weeks)	N.S.	N.S.	N.S.	N.S.	N.S.
Low birthweight	N.S.	↑	N.S.	↓	↓
Female	↓	N.S.	N.S.	N.S.	N.S.
Environmental Characteristics					
Increasing Temperature	↓	↓	↓	↓	↑
Increasing Humidity	↑	↑	↓	N.S.	↑

N.S.: Not statistically significant

Figure 4-1: Study Flow Diagram



*20% of full term neonates were randomly selected for inclusion and all preterm neonates were selected for inclusion in the sub-study.

Figure 4-2: TEWL During Neonatal Period

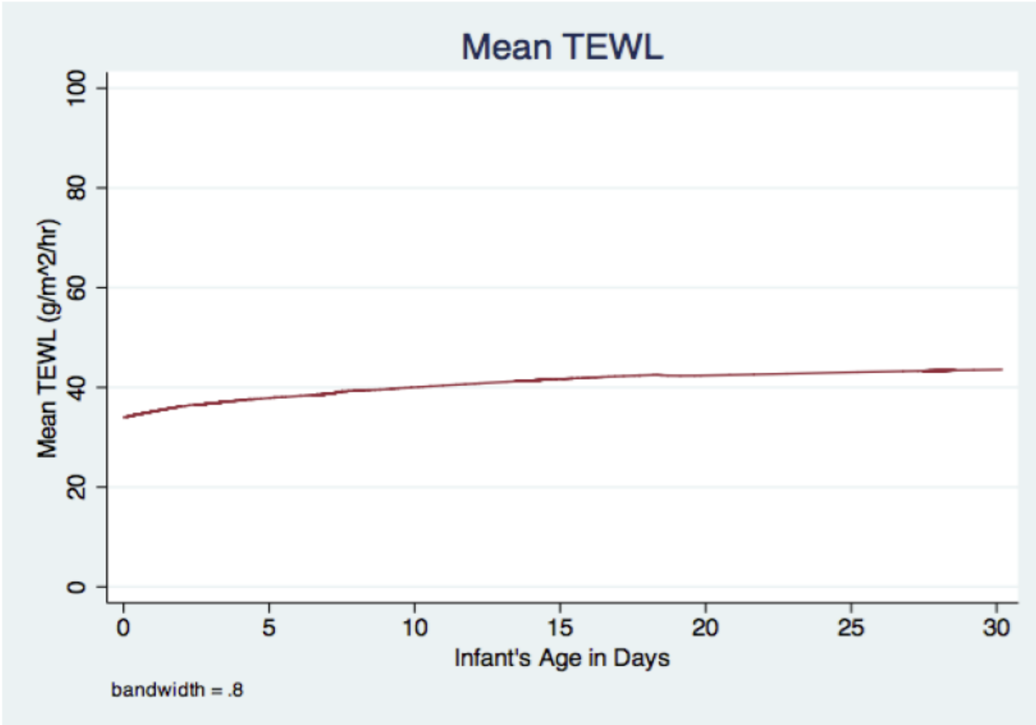


Figure 4-3: Skin pH During Neonatal Period

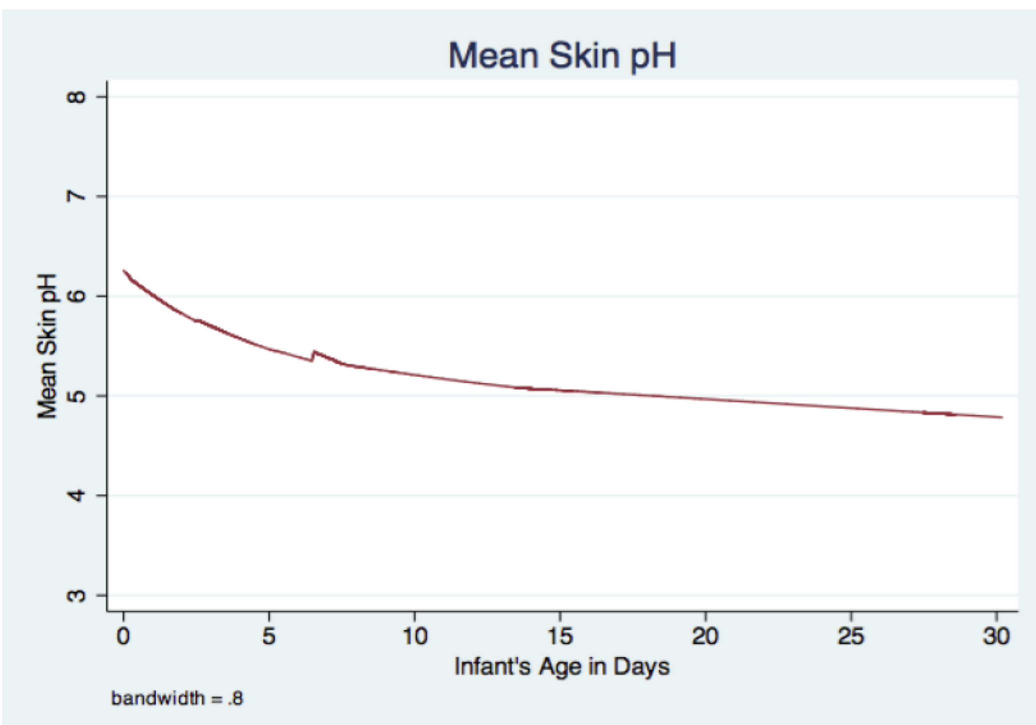


Figure 4-4: Stratum Corneum Protein Concentration During Neonatal Period

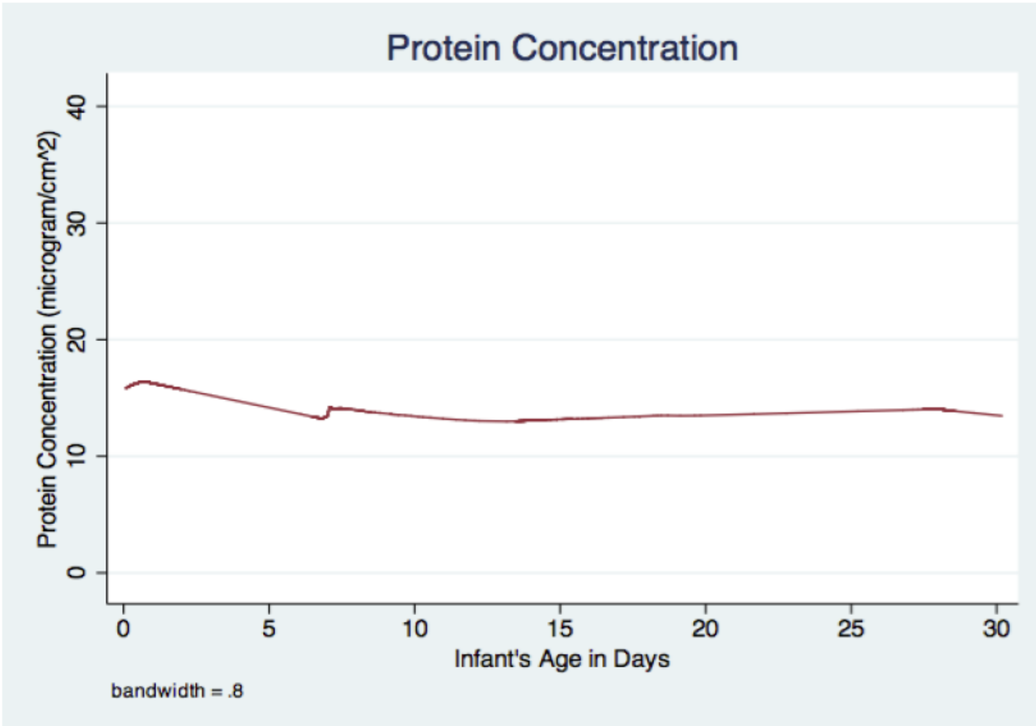
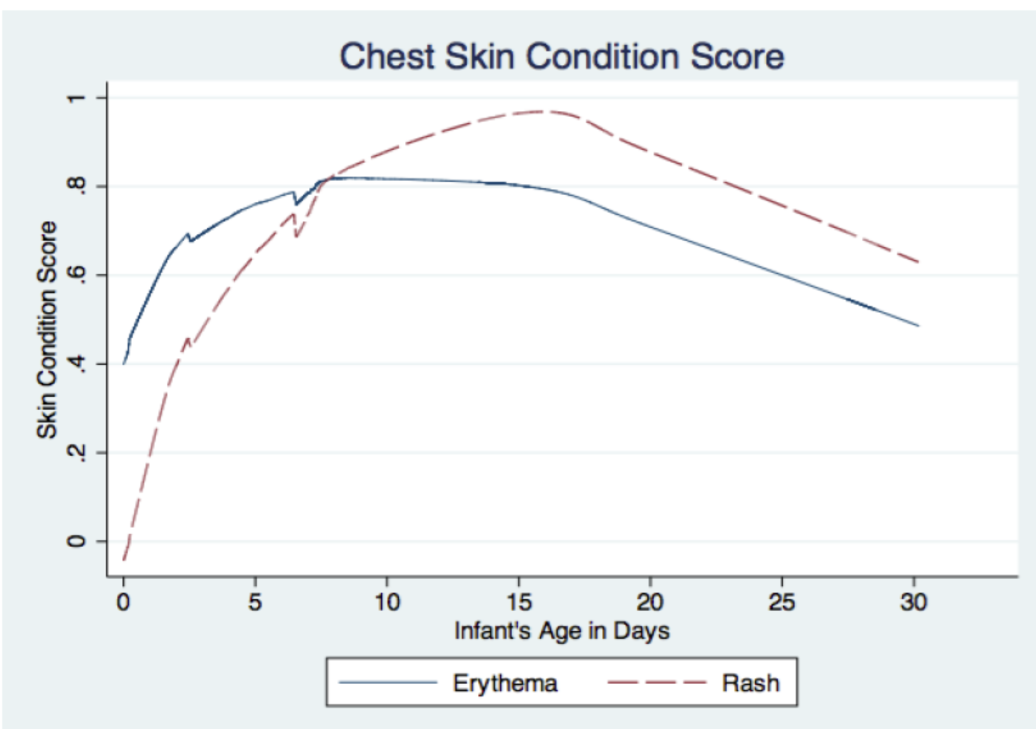


Figure 4-5: Chest Skin Condition Score During Neonatal Period



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Chapter 5 Effect of Oil Group on Skin Integrity and Barrier Function

Background

Ninety-eight percent of the 3.1 million neonatal deaths occurring each year occur in developing countries. (Black, Cousens et al. 2010; Lawn, Kerber et al. 2010; Liu, Johnson et al. 2012) The top three causes of death in these infants are complications from preterm birth (35%), infections (including sepsis, pneumonia, diarrhea, meningitis, and tetanus) (27%), and intrapartum related neonatal deaths, such as birth asphyxia (23%). (Liu, Johnson et al. 2012) In settings with high neonatal mortality (NMR), infections account for about 50% of deaths. (Lawn, Cousens et al. 2005; Blencowe and Cousens 2013) In Southeast Asia, 52% of child deaths occur during the neonatal period and in Nepal it is estimated, 58% of under-five deaths occurred during this period in 2010. (Liu, Johnson et al. 2012)

One intervention being explored to reduce neonatal mortality is the practice of neonatal oil massage using locally available vegetable oils, which is practiced almost universally throughout Southeast Asia. Studies in Bangladesh and Nepal found that more than 96% and 99% of newborns, respectively received full body oil massage, with mustard seed oil being the most commonly used oil in both settings. (Darmstadt and Saha 2002; Mullany, Darmstadt et al. 2005) Newborn oil massage is also commonly practiced in many communities in Africa. (Iweze 1983; Niang 2004; Darmstadt, Hussein et al. 2007; Mrisho, Schellenberg et al. 2008; Waiswa, Nyanzi et al. 2010; Duffy, Ferguson et al. 2012).

Many studies show that massage with oil is more beneficial than massage alone. These studies show that infants massaged with oil displayed fewer stress behaviors, lower salivary cortisol levels, increased vagal activity, and greater mean weight gain when compared with infants massaged without oil. (Field, Schanberg et al. 1996; Arora, Kumar et al. 2005; Fallah, Akhavan Karbasi et al. 2013; Kumar, Upadhyay et al. 2013) In addition, hospital-based studies show that the application of sunflower oil reduces rates of invasive infection and mortality. A study in Egypt of preterm infants <34 weeks showed a significant decrease in the incidence of nosocomial infections and an improvement in skin condition in infants receiving sunflower oil. (Darmstadt, Badrawi et al. 2004) Another study of preterm infants in Bangladesh comparing infants randomized to massage with sunflower oil, Aquaphor, or no emollient, found a 41% reduction in nosocomial infections (Darmstadt, Saha et al. 2005) and a 26% reduction in neonatal mortality rates in the sunflower oil group compared with controls. (Darmstadt, Saha et al. 2008)

Although studies have shown that topical application of vegetable oil may be beneficial to neonates, the choice of oil may be important. A study done using a mouse model showed accelerated skin barrier recovery in mice one hour after application of sunflower oil, with a sustained effect up to five hours. (Darmstadt, Mao-Qiang et al. 2002) Conversely, application of mustard, olive, or soybean oils showed delayed recovery of skin barrier function when compared to untreated or Aquaphor treated skin. The most detrimental effects were seen with mustard seed oil, with sustained delay of barrier recovery for up to seven days. (Darmstadt, Mao-Qiang et al. 2002) Another study showed topical application of olive oil on adult forearms for 4 weeks caused significant reduction in skin integrity and induced mild erythema, while sunflower oil preserved skin integrity and improved hydration. (Danby, AlEnezi et al. 2013)

Newborn skin is the first line of defense between the infant and its environment and serves many important functions. These include: moderating fluctuations in transepidermal water loss (TEWL) and maintaining electrolyte homeostasis, thermoregulation and minimizing caloric losses, antimicrobial defense, protection from environmental toxins, protection from ultraviolet (UV) radiation, and tactile stimulation. At birth, full term infant skin is fully functional with a thick epidermis and well-formed stratum corneum layers. (Cunico, Maibach et al. 1977; Yosipovitch, Maayan-Metzger et al. 2000) Preterm infants however, show immature barrier function. (Evans and Rutter 1986; Cartlidge 2000; Darmstadt and Dinulos 2000; Rutter 2000) In addition, neonatal skin is always adjusting to its extrauterine environment as it adapts to its new cooler, less humid surroundings. (Visscher, Chatterjee et al. 2000; Chiou and Blume-Peytavi 2004; Fluhr, Darlenski et al. 2012; King, Balaji et al. 2013)

Improving skin barrier integrity and function may be one way in which emollient therapy with vegetable oil may improve the health of newborns. Emollient therapy using sunflower or safflower oil may normalize transepidermal water loss, which is related to gestational age, as TEWL has been shown to decrease in infants treated with emollient therapy. (Darmstadt and Dinulos 2000; Hoath and Maibach 2003; Nangia, Paul et al. 2008) It could also improve skin condition directly providing metabolic building blocks to the stratum corneum to form healthy layers and repair damaged layers (Elias, Brown et al. 1980; Mao-Qiang, Elias et al. 1993). Improved skin barrier integrity could also provide fewer portals of entry of invasive pathogens, leading to fewer invasive infections. (Nopper, Horii et al. 1996; Edwards, Connter et al. 2004; Darmstadt, Saha et al. 2005)

The acidic pH of the skin is another mode of protection and is important in the formation and integrity of the stratum corneum, allowing the effective functioning of enzymes that are involved in lipid metabolism, bilayer structure, ceramide synthesis, and desquamatization. (Rippke, Schreiner et al. 2002; Schmid-Wendtner and Korting 2006) After birth, skin pH is elevated in full term and preterm neonates compared with adults and older children and decreases in the first few weeks, with the most significant reductions occurring during the first 4 days of life. (Green, Carol et al. 1968; Fluhr, Pfisterer et al. 2000; Visscher, Chatterjee et al. 2000; Hoeger and Enzmann 2002) However, skin pH remains higher for longer in very premature infants. (Fox, Nelson et al. 1998) A study looking at skin care practices in a Neonatal Intensive Care Unit (NICU) found that using skin wipes with emollient cleansers resulted in significantly lower skin pH levels compared with using a cloth and water. (Visscher, Odio et al. 2009)

Emollient therapy research to improve neonatal survival is a global priority. (Lawn, Zupan et al. 2006) Although millions of infants in South Asia are massaged with mustard oil each year, it is unknown whether substituting sunflower seed oil changes any biological mechanisms that may improve neonatal health. With massage of neonates with mustard seed oil being almost universally practiced in many communities in Southeast Asia, neonates are routinely exposed to the effects of mustard oil massage, which may include increased risk of microfissures in the epidermal layer that may increase the likelihood of transcutaneous acquisition of invasive pathogens from the environment. (Darmstadt and Dinulos 2000; Darmstadt, Saha et al. 2003) We aimed to determine how and if certain biological mechanisms relating to skin barrier and integrity including skin condition (measured visually as rash and erythema), skin pH, TEWL, and stratum corneum protein concentrations (measured instrumentally) might differ between infants massaged with mustard seed oil and sunflower seed oil in rural Nepal.

Methods

Settings and Population

This was a cluster-randomized community-based trial conducted by the Nepal Nutrition Intervention Project, Sarlahi (NNIPS). In 2011, the NNIPS surveillance area consisted of 26 Village Development Committees (VDCs) each encompassing nine government defined geopolitical units (wards), which were further divided into sectors based on population. This trial was nested within a larger parent trial on the Impact of Sunflower Seed Oil Massage on Neonatal Morbidity and Mortality in Nepal (NOMS). The study population for the NOMS trial was all live born infants in households in 13 VDCs in Sarlahi District in rural Nepal. The Nepal Health Research Council and the Committee on Human Research of the Johns Hopkins Bloomberg School of Public Health approved this study.

Procedures and Design of the Parent Trial

Previous sector level mortality estimates were known for 9 of the 13 VDCs from prior research in this community and were used to perform restricted randomization in order to ensure balance of prior neonatal mortality risk. In the other 4 VDCs, sectors (clusters) were randomized with a computerized quasi-random number generator, stratified on VDC using blocks of 4, ensuring a geographical balance of the types of oil across the study area. Newborn infants were randomized within clusters (sectors) to receive either promotion of full body massage with sunflower seed oil or promotion of full body massage with mustard seed oil. A cluster-randomized design was chosen in order to minimize the chance of crossover or contamination of the intervention by providing each field worker with only one type of oil to promote in her area. It was not possible to blind field workers and mothers to the type of intervention as mustard oil and sunflower oil have distinct colors and smells.

For both intervention and control groups, oil was purchased from Shiv Shakti in Jitpur, Nepal, approximately every 4-6 weeks and stored in sealed half-liter plastic packets at site headquarters at room temperature. The company was required to submit a sample for purity/quality to the Government of Nepal Food Inspection Laboratory in Hetauda, Makwanpur District, Nepal. A copy of this analysis was received from the Hetauda food lab for each distinct batch. Determination of the fatty-acid composition of the oils was done at Geo-Chem Laboratories PVT LTD (Mulund, Mumbai, India). This fatty-acid profile analysis was repeated twice per year. These analyses consistently estimated the linoleic acid levels between 58%-67% and 18%-22% in the sunflower and mustard oils, respectively.

Community-specific lists of married women of reproductive age were created. In order to rapidly identify new pregnancies, locally-resident female workers monitored the status of these women every 5 weeks. A woman identified as pregnant was approached for recruitment and consent for the infant's participation in the parent trial. Each woman participating in the study received a set of basic antenatal care interventions (e.g. tetanus toxoid, a clean delivery kit, iron-folate supplements, chlorhexidine cleaning solution for disinfecting the umbilical region, iron folate, and deworming) and basic educational messages on antenatal and essential newborn care. The worker visited the women in late pregnancy (~28-32 weeks) to promote the use of either the sunflower seed oil or mustard seed oil and provide the mother or other caretaker with a 100ml bottle of oil at that time. The mother or other caretaker initiated full body massage following the field worker's guidelines using the provided oil daily throughout the newborn period. The local project worker visited the homes daily during the first week of life to promote the continued use of the oil.

Immediately upon notification of the pregnancy, the local resident worker notified the supervisory staff and a birth assessment was conducted using standardized data collection forms recording information relating to late pregnancy morbidity, labor and delivery characteristics, birth assistants and practices, length of labor, maternal temperature, date and time of birth, sex, length and weight of infants, and immediate newborn care practices (thermal care, cord practices, early bathing and massage, breastfeeding initiation, etc.). In addition to the individual data obtained, household level data were collected at the time of enrollment of the mothers. This included information on socioeconomic factors, such as ethnicity, caste, household assets, ownership of materials or livestock, water sources, and family members who may be working overseas. Data were also collected on parental literacy, education, and birth history. After the initial birth visit, the birth team member completed newborn follow-up visits (NFF) on days 3, 7, 10, 14, 21, and 28 (for a total of 7 visits), where signs of infant morbidity and newborn care practices since prior visit were recorded. A 500ml bottle of oil was provided at the initial visit and on follow-up visits on days 10 and 21. The primary outcomes of NOMS are mortality within 28 days of birth, and possible severe infection in newborns.

Design of the Biological Mechanisms Sub-Study

This biological mechanisms sub-study included an extended set of measurements collected from a subset of infants participating in the main trial. The focus of data collection for this sub-study was on direct measurement of biological markers of infants built into a specific sub-study schedule (days 1, 3, 7, 14, and 28) of the parent trial visits. The biological measurements used to assess skin integrity included: visual assessment of skin condition (erythema, rash), measurement of transepidermal water loss (TEWL), measurement of skin pH, and collection of skin discs in order to measure the stratum corneum cohesion and protein concentration via measurement of optical density. All skin integrity measures were taken at each of the five visits, apart from the skin disc collection, which was collected at visits 1, 7, 14, and 28. Table 5-1 shows the timing of measurement collections.

A subset of 7 VDCs of the 13 original VDCs in the NOMS trial was selected to participate in the mechanisms study, which began in July 2012. An 8th VDC was added after 5 months and a 9th VDC was added in January 2013. The cluster-randomization of the parent trial was conserved for the mechanisms study. Among infants participating in the main study, determination of eligibility to additionally participate in the sub-study was done during the initial birth assessment visit, and was based on estimates of gestational age at birth. For this study, preterm infants (<37 weeks gestational age) were oversampled, aiming for approximately 50% of the sample to be preterm. During the study period (July 2012 until September 2013), the gestational age was estimated directly by the field worker using the date of last menstrual period estimated by the woman at the time of initial enrollment. Each birth team member had a list of the date of the mother's last menstrual period (LMP), which was used along with the date of birth to determine the gestational age of the infant. If the infant was born before week 37, one of the members of a specially trained team of field workers focused on the implementation of the mechanisms sub-study was contacted directly by mobile phone. Infants born on or after week 37 were listed by VDC in consecutive order (i.e. by birth date) on a pre-printed computer-generated blank form of 20 rows, 4 of which had been randomly selected for shading. Infants listed on a randomly shaded row were eligible for inclusion in the mechanisms study, thus selecting 4 out of every 20 (20%) of the full term infants for participation. All preterm (<37 weeks) infants and every 5th full term infant born in the mechanisms study area were eligible for enrollment in the sub-study. Infants were enrolled if consent was provided and the infant was met alive within 48 hours after birth.

Newborns enrolled in the mechanisms study were visited in their homes 5 times, on days 1, 3, 7, 14, and 28. In addition to performing the skin integrity measurements at these visits, the workers asked questions relating to newborn care practices since the prior visit, including breastfeeding and other feeding practices, bathing, and massage practices.

Measurement Methods

Four measures were taken to evaluate skin integrity: skin condition scores, TEWL, skin pH, and stratum corneum protein concentration (via optical density). Field worker evaluation of skin condition was measured using a modified version of a scoring method described by Visscher et al. (Visscher, Odio et al. 2009) This team has experience extending these scores for patients in the United States. This study used an extended version of more established scales for the measure of irritation of the skin based on levels of erythema and rash, rating erythema severity on a scale from 0 to 3 and area affected on a scale from 0 to 4. Severity of rash and area affected by rash were evaluated on scales from 0 to 4 and 0 to 6 respectively. Scores were based on the percent area of involvement and severity of skin compromised within the specified body region for each component. Each field worker was trained in the use of the scales. The severity of erythema and rash as well as the area covered was recorded on standardized data collection instruments. Skin condition assessments were completed on the same 4cm by 4cm area of the chest, the entire left arm, and the entire right leg. Transepidermal water loss was measured in $g/m^2/hr$ using a VapoMeter (Delfin Technologies, Ltd, Finland), a closed-chamber device containing sensors for relative humidity and temperature, using standardized procedures. (Rogeries and Group 2001; Nuutinen, Alanen et al. 2003; De Paepe, Houben et al. 2005) At each visit, transepidermal water loss measurements were taken three times at the mid-chest nipple line. The mean value of these measurements was used for analyses. The exact location where measurements were taken was similar in all infants through the measurement of anatomical markers. The location of the measurements was chosen because it was easily accessible, an area that was massaged, and provided a reasonably flat surface area for placing the VapoMeter. The three measurements along with the temperature and relative humidity obtained from the VapoMeter were recorded on standardized data collection forms.

Measurements of skin pH were performed with a flat electrode (Skincheck™, Hanna Instruments, UK) calibrated daily to pH 4 and 7. (Parra and Paye 2003) Measurements were taken in the same location at the mid-nipple as the TEWL measurements. The three measurements were recorded on standardized data collection forms and the mean skin pH of the three measurements was used for analyses.

Using a technique described by Voeglei et al., protein content from the outermost stratum corneum was analyzed using samples collected using 380 mm^2 D-Squame adhesive discs (CuDerm, TX) applied to the chest with constant pressure for 2 minutes. (Voegeli, Heiland et al. 2007) Different locations on the chest were used for the placement of the skin discs at each of the four visits in order to collect discs from skin that had not been previously exposed to adhesive. These locations were different from where the skin pH and TEWL were measured. After the discs were removed from the chest, they were placed in microtubes and transferred to field headquarters in cold boxes kept between 2° and 8°C. At field headquarters, optical absorption of the skin discs was determined using a spectrophotometer SquameScan™ 850A (Heiland electronic, Wetzlar, Germany) specifically designed for the application of D-Squame discs. Before the optical absorptions of the discs were read, the spectrophotometer was calibrated to 0% and 36.2% optical absorption. Optical absorption of the discs was recorded on standardized data collection forms. The following equation was used for quantification of protein concentration: (Voegeli, Rawlings et al. 2007)

$$C_{protein}\left(\frac{\mu g}{cm^2}\right) = 1.366 * Absorption(\%) - 1.557$$

Statistical Analysis

The nested biological mechanisms study included a subset of the sample size of 29,620 infants required for the parent trial. Given that we hypothesized that the outcome measures (and the moderating effect(s) of the different oils, if any) may be more important in preterm infants than full term infants due to the immaturity of their skin, preterm infants were oversampled. A total sample size of 1000 infants was selected, equally stratified by 500 preterm and 500 full term infants. All power calculations assumed a design effect of 1.5, a 5% loss to follow-up, and a 5% Type 1 error. A design effect of 1.5 was assumed without prior knowledge of the true extent of correlation of the different measurements within clusters. The chosen sample size of 1000 infants enabled detection of a difference in mean values of skin condition scores, skin pH, TEWL, and protein concentrations of 0.5 with between 71% and 100% power for standard deviations of 1.0 to 2.5. In addition, the sample size provided 80% power to detect a true hazard ratio of 20% in a Cox proportional hazards analysis of the time required for newborn skin to reach a beneficial pH level of 5.7. (Yosipovitch, Maayan-Metzger et al. 2000) The analyses presented here represent a preliminary analysis of the first 63.7% of the anticipated enrolled infants.

All analyses for these measurements were conducted using STATA v12 (College Station, TX). The comparability of the treatment groups in relation to their background characteristics was assessed to determine if there were any confounding variables. Possible confounders included: household demographics and SES, ethnic group, caste, maternal and paternal education levels, maternal age, reproductive history, labor and delivery characteristics (place of birth, length and type of labor, type and practice of birth assistants), newborn characteristics and newborn care practices (sex, birthweight, gestational age, small-for-gestational age (SGA) status, breastfeeding), and intervention exposure and compliance. Outliers that were biologically impossible were removed from the analyses on the basis that they were likely due to an instrument malfunction or measurement of another biological process (e.g. measurement of perspiration rather than TEWL). Skin pH values greater than 8 and less than 3 (Marty Visscher, personal communication) were removed as well as TEWL values greater than 100 $g/m^2/hr$ (Aki Immonen, personal communication). A total of 22 (<1%) skin pH measurements were removed, although due to using the mean of 3 measurements, this did not result in any lost observations. Thirty-nine (1.3%) TEWL observations were removed as a result of 133 (4%) measurements being out of range.

In order to analyze differences in skin pH, the differences in the time it took for infants in each group to reach a beneficial skin pH level (<5.7) was assessed using Cox regression models. (Yosipovitch, Maayan-Metzger et al. 2000) For this survival analysis, infants were censored at the earliest of: skin pH = 5.7 (event), death, loss to follow-up, and after 28 days.

Skin condition scores for erythema and rash were added together separately for each body region assessed to get a total skin condition score for each region (chest, left arm, and right leg). To examine whether there were differences in skin condition, TEWL, skin pH, and stratum corneum protein concentration at different follow-up visit times between the different oil groups, regression analyses with random intercepts were performed. In all models, estimates of standard errors accounted for the clustered design. Regression models were also run for each of the skin condition score components (erythema and rash) to examine whether there was a difference between the two oil groups for either component score. In addition, mixed-effects, multi-level regression models with random intercepts accounting for the randomized clusters and repeated measures on each infant were performed for the full neonatal period and stratified by early (<7 days) and late (≥7 days) neonatal periods. Mixed-effects, multi-level models with an interaction between infant's age and oil group were also used to explore whether the rate of change in skin pH, TEWL, or stratum corneum protein concentration differed between the two oil groups. Linear splines for infant's age at measurement were used where needed to account for nonlinearity, assessed through locally weighted regression smoothing. Sub-group analyses were conducted for preterm infants for each measurement. Analyses followed an intention-to-treat approach for participating infants, regardless of the actual treatment provided.

Results

Between July 23, 2012 and September 30, 2013, there were a total of 1,876 full term and 409 preterm live born infants in the study area that were eligible for enrollment in the biological mechanisms study. Of these infants, 785 (376 full term and 409 preterm) were selected for enrollment (Figure 5-1). Of those selected, 148 (18.9%) died or were not met by the mechanisms study field worker before 48 hours after birth and 5 (0.6%) mothers refused their infant's participation in the study. A total of 632 newborns were enrolled in the mustard seed oil (N=324) and sunflower seed oil (N=308) clusters. In total, 271 (83.6%) and 262 (85.1%) newborns completed 28 days of follow-up in the mustard seed oil and the sunflower seed oil groups respectively. In the sunflower oil group, 7 (2%) infants died and 39 (13%) permanently moved, most likely returning to the mother's maternal home or *maiti*, or were not met at their 28-day visit. In the mustard oil group, 6 (2%) infants died and 47 (14%) permanently moved or were not met at their 28-day visit.

Socioeconomic, household, and individual characteristics were well balanced between groups (Table 5-2). In the sunflower seed oil group, a greater proportion of infants were born to families who were of *Pahadi* (originating from the hills) ethnicity than in the mustard oil group. Households in the sunflower seed oil group were less likely to have electricity and were more likely to have low birthweight (<2500g) infants. In the sunflower seed oil group, 40.3% of infants were low birthweight compared to 33.9% in the mustard seed oil group. Overall prevalence of low birthweight in this study population with oversampled preterm births was 36.7%. Both oil groups had similar proportions of infants that were small-for-gestational age (SGA). The overall prevalence of infants born SGA was 42.7%. The mustard seed oil group had a slightly higher proportion of infants who were born prematurely with 45.4% of newborns in the mustard seed oil group and 39.9% of newborns in the sunflower seed oil group born premature. Overall prevalence of prematurity in the study population was 42.7%. As these analyses were not completed on the total sample size, recruitment was still ongoing. Premature infants were being enrolled at a slower rate than full term infants, as our estimated proportion of preterm infants in this study (20%) was based on data from older studies. The actual proportion of infants who were born preterm in the study area during this study period was closer to

16%, accounting for the prevalence of preterm infants in the population used for these analyses being less than 50%.

The number of follow-up visits was similar for both treatment groups (see Table 5-3). Both groups had very high percentages of newborns that had been massaged since the previous visit (or since birth, if visit 1). The sunflower seed oil group had a larger proportion of infants who were not massaged with the study provided oil. Both groups had the lowest proportion of infants massaged with the study provided oil during their last massage prior to visit 1 (92.3% in the mustard oil group and 82.5% in the sunflower oil group), although greater than 99% of infants were massaged prior to the first visit. Both groups were massaged frequently during their first week of life, with infants in the mustard oil group massaged slightly more frequently, with a mean of 32.34 (±10.78) massages in the first week compared to 29.79 (±9.36) massages during the first week in the sunflower oil group, The average number of massages per day was 4.70 (±1.47) and 4.31 (±1.27) in the mustard oil group and the sunflower oil group, respectively. The time between last massage and follow-up visits was similar in both treatment groups.

Effect of Oil Group on TEWL

In the complete data set TEWL of both oil groups showed an increasing trend over time, however this leveled off in the sunflower oil group after day 3. Both the mustard oil group and sunflower oil group show a trend of increasing mean TEWL over time in the full term group, whereas preterm infants in the sunflower oil group had mean TEWL increasing quickly from visits 1 to 3 and decreasing after visit 3. Figures 5-2 and 5-3 show scatterplots of the mean TEWL at each visit with weighted linear least squares regression curves for each oil group for the complete data and the preterm group respectively. (Full term group figures for all measurements are shown in Appendix B.) In this population, neonates in the full term groups had similar mean TEWL measures at most visits (Table 5-4). However in the preterm group, the sunflower oil group had slightly higher mean TEWL at visit 1 (35.52 $g/m^2/hr$ (±24.19)) than the mustard oil group (31.60 $g/m^2/hr$ (±21.64)). At visit 3, the sunflower oil group's TEWL in the preterm infants was higher compared with the mustard oil group (41.49 $g/m^2/hr$ (±25.89) compared with 33.81 $g/m^2/hr$ (±23.85)). However, after visit 3 the sunflower oil group's TEWL starts decreasing, whereas the mustard oil group's TEWL continues to increase. Mean TEWL measures at visit 28 for the preterm groups were 38.09 $g/m^2/hr$ (±21.88) versus 43.63 $g/m^2/hr$ (±24.19) for sunflower oil and mustard oil, respectively.

Table 5-5 shows the results from the regression models, indicating that the sunflower oil group had a statistically significantly higher mean TEWL value at visit 3 for the preterm data set. It is estimated that the mean TEWL in the sunflower oil group was 7.27 $g/m^2/hr$ (95% CI: 0.65-13.89) higher when compared to the mustard oil group in preterm infants at visit 3. There were no other statistically significant differences of the mean TEWL values at any of the other visits when comparing the sunflower oil group to the mustard seed oil group for the complete data set or when stratified by term status. The results indicate there was no effect of oil group on TEWL over the complete neonatal period or when stratified by early and late neonatal periods.

A statistically significant difference was found in the rate of change in mean TEWL over time in the sunflower oil group when compared with the mustard oil group. The sunflower oil group's mean TEWL decreased 0.16 $g/m^2/hr$ (95% CI: 0.016-0.30) per day faster over the 28-day period than the mustard oil group in the complete data set (i.e. without regard to preterm status). Investigating different time periods within the 28-day period showed no statistically significant difference in the rate of change of mean TEWL from day 0 to day 3. However, from day 4 to day 28 the sunflower oil group's TEWL decreased an estimated 0.23 g/m^2/hr (95% CI: 0.06-0.39) per day more quickly than the mustard seed oil group's TEWL in the complete data set. The preterm group showed a similar result, with the sunflower oil group's mean TEWL decreasing at an estimated rate of 0.39 g/m^2/hr (95% CI: 0.18-0.61) faster than the mustard oil group for the complete 28-day period and 0.47 g/m^2/hr (95% CI: 0.22-0.72) per day faster between days 4 and 28.

Effect of Oil Group on Skin pH

In this population, neonates in the sunflower oil group had a higher mean skin pH on visit 1 for the complete set of neonates and when stratified by term status when compared to the mustard oil group (Table 5-6). The mustard oil group had means of 5.95 (±0.51), 5.98 (±0.51), and 5.93 (±0.51) for the complete data, the full term group, and the preterm group, respectively. The sunflower oil group had means of 6.12 (±0.53), 6.15 (±0.56), and 6.09 (±0.48) for the complete data, full term group, and preterm group, respectively. During the subsequent visits the means were more similar between groups. Figures 5-4 and 5-5 show how skin pH changes over the complete neonatal period for each oil group for the complete data set and preterm group, respectively. These graphs further illustrate the difference in mean skin pH values for the sunflower and mustard oil groups at visit 1. The graphs also show a trend of decreasing mean skin pH values over time for both the sunflower oil group and the mustard seed oil group for the complete data and when stratified by term status.

The regression models indicate that the sunflower oil group had a statistically significant higher mean skin pH value at visit one for the complete data set, the full term group, and the preterm group (Table 5-7). For the complete data, it is estimated that at visit 1 the mean skin pH in the sunflower oil group was 0.16 (95% CI: 0.07-0.25) higher when compared to the mustard oil group. When stratified by term status, it is estimated that the mean skin pH in the sunflower oil group was 0.17 (95% CI: 0.06-0.28) higher in the full term group and 0.16 (95% CI: 0.03-0.28) higher in the preterm group when compared to the mustard oil group. There were no other statistically significant differences in mean skin pH values at any of the subsequent visits when comparing the sunflower oil group to the mustard seed oil group. During the early neonatal period the mean skin pH in the sunflower oil group was estimated to be 0.12 (95% CI: 0.04-0.20) higher than the mustard seed oil group in the complete data set and 0.12 (95% CI: 0.02-0.21) and 0.13 (95% CI: 0.03-0.23) higher in the full term and preterm groups, respectively. There were no significant effects on mean skin pH when comparing the different oil groups for the complete or late neonatal periods.

A statistically significant difference was found in the rate of change in skin pH over time in the sunflower oil group when compared with the mustard oil group. The sunflower oil group's mean skin pH decreased 0.006 (95% CI: 0.003-0.01) per day more quickly than the mustard oil group over the 28-day period for the complete data set. The same trend was seen in the full term group, with the sunflower oil group's mean skin pH decreasing 0.007 (95% CI: 0.002-0.03) per day more quickly when compared with the mustard oil group over the 28-day period. The preterm group showed no statistically significant difference in the rate of change of the mean skin pH over the 28-day time period when comparing the two oil groups, although it showed a trend of decreasing at a faster rate, which was similar to the full term and complete data set groups. During the first week after birth, the faster rate of decrease was even more pronounced for the complete data set, with the sunflower oil group's skin pH decreasing 0.018 (95% CI: 0.003-0.033) more quickly than the mustard oil group's skin pH. The full term and preterm groups did not show any significant differences in rates of decrease when stratified by early and late neonatal periods.

Cox regression models (adjusted for first skin pH measure) were also fit for the data to investigate if there were differences in the hazard of the mean skin pH reaching a value of 5.7 when comparing the sunflower oil group to the mustard oil group. At day 7, 235 (73%) infants in the mustard oil group had reached a skin pH of 5.7 compared with 261 (70%) infants in the sunflower oil group. However, by day 28 the proportion of infants in the sunflower oil group reaching a skin pH of 5.7 surpassed that in the mustard oil group with 298 (97%) infants in the sunflower oil group reaching a pH of 5.7 compared with 305 (94%) in the mustard oil group. The models did not show statistically significant differences in the hazards of reaching a skin pH of 5.7 in the complete data set or when stratified by term status (Table 5-8). Figures 5-6 and 5-7 show the Cox proportional hazards regression survival curves for the complete data set and the preterm group.

Effect of Oil Group on Stratum Corneum Protein Concentration

Overall, both groups showed a decrease in protein concentration from visit 1 to visit 7 with a leveling off from visits 7 to 14 and then a slight increase between visits 14 and 28 (Figures 5-8 and 5-9). Table 5-9 shows the means and standard deviations for each group by visit. The mustard oil group had means of 16.07 μg/cm^2 (±8.14), 12.45 μg/cm^2 (±6.26), 12.89 μg/cm^2 (±6.12), and 13.68 μg/cm^2 (±6.26) and the sunflower oil group had means of 16.75 μg/cm^2 (±7.61), 13.40 μg/cm^2 (±6.52), 13.20 μg/cm^2 (±6.52), and 14.36 μg/cm^2 (±5.86), for the complete data set on visits 1, 7, 14, and 28 respectively. When stratified by term status, the full term and preterm groups showed similar results with very similar means between the two oil groups, except on visit 7 in the full term group where the sunflower oil group had a higher mean protein concentration of 13.54 μg/cm^2 (±6.40) compared with the mustard oil group's mean of 11.70 μg/cm^2 (±6.26).

The regression models indicate that the only visit where mean protein concentration was statistically significantly different between oil groups was in the full term group on visit 7 (Table 5-10). Comparing sunflower oil to mustard oil in full term infants on visit 7, the sunflower oil group is estimated to have 1.84 μg/cm^2 (95% CI: 0.37-3.31) higher protein concentration than the mustard oil group. Regression models for the complete neonatal period indicated mean protein concentrations in the sunflower oil group for full term infants to be 0.97 μg/cm^2 (95% CI: 0.07-1.87) higher than the mustard oil group, however this was no longer significant when adjusted for whether the household had electricity. When stratified by early and late neonatal periods full term infants in the sunflower oil group had protein concentrations 1.26 μg/cm^2 (95% CI: 0.15-2.38) higher during the late neonatal period compared with the mustard oil group (these results did not change when controlling for confounders). The rate of change of the protein concentrations comparing the two oil groups was not different in the complete data set or when stratified by term status for any time period over the first 28 days.

Effect of Oil Group on Skin Condition

Neonates in this population had similar chest skin condition scores for each oil group at all visits for both the complete group of neonates and when stratified by term status. This was observed for the total skin condition score and for erythema and rash individually (results not shown). The total chest skin condition score showed an increasing trend until around day 17, followed by a decreasing trend until day 28, observed in both the complete data set and when stratified by term status (Figures 5-10 and 5-11). This trend was also observed for each component of the skin condition score for both the mustard seed oil and the sunflower seed oil groups (Figure 5-12). Table 5-11 shows the mean skin condition scores for each component, along with the mean total skin condition score. Both rash and erythema increased until visit 14 and then decreased between visit 14 and visit 28 in the complete data set and when stratified by term status (Tables 5-11, 5-12, and 5-13).

The regression model indicates no statistically significant differences between total chest skin condition scores between oil groups at any of the follow-up visits for the complete data set or when stratified by term status (Table 5-14). In addition, regression models for rash and erythema individually showed no statistically significant differences for any of the component scores for the complete data or when stratified by term status for any of the follow-up visits (data not shown). Infants in the sunflower oil group had a trend of lower skin condition scores during the neonatal period compared with the mustard oil group for the complete data set and when stratified by term status, although this was not statistically significant. Stratification of the regression models by early and late neonatal periods also suggested that the type of oil group had no effect on the total skin condition score of the chest, nor did it have an effect on any of the skin condition score components for either of the period stratifications. This was true for the complete data set and when stratified by term status.

Scatterplots, descriptive characteristics, and regression results of skin conditions for the left arm and right leg are shown in Appendix B. These regions showed similar results to the chest region. There was an increasing trend in mean total skin condition and for erythema and rash individually in the left arm and right leg region until around day 16 and then a decreasing trend until day 28, which was similar in both oil groups. The only visit showing a statistically significant difference in skin condition scores between oil groups was for the left arm at visit 1, which showed the sunflower oil group to have an estimated skin condition score 0.24 (95% CI: 0.03-0.45) lower than infants in the mustard oil group after adjusting for birthweight and whether the household had electricity (results not shown). Similarly to the chest region, infants in the sunflower oil group tended to have lower skin condition scores during the neonatal period for the complete data set and when stratified by term status for both the left arm and the right leg, although this was not statistically significant (Appendix B).

Discussion

These data provide some evidence that different types of oil may contribute to differences in skin integrity measurements when infants are massaged regularly with sunflower seed oil compared with mustard seed oil. The mean skin pH in the sunflower oil group decreased at a faster rate in the complete data set (0.006 pH units per day) and in the full term group (0.007 pH units per day) over the 28-day follow-up period. The skin pH of infants in the sunflower seed oil group was higher than in the mustard seed oil group at visit 1, resulting in the early neonatal period showing a statistically significant difference in the mean skin pH in the sunflower oil group. Due to the fact that the sunflower oil group started with a higher mean skin pH at visit 1 this difference is unlikely an effect of the different oils over the first week of life. This faster decrease in skin pH could indicate improved barrier function in the sunflower oil group caused by a more quickly forming acid mantle, which is important in order for the skin to maintain bacteriological, chemical, and mechanical resistance. (Schmid-Wendtner and Korting 2006)

The values of mean TEWL in this population started much higher than in other populations where TEWL has been measured and increased during the first month of life. This is in contrast to other studies that have found TEWL measures in full term infants to be lower than or equal to adults and to remain relatively stable during the first month of life, indicating a highly effective skin barrier. (Cunico, Maibach et al. 1977; Yosipovitch, Maayan-Metzger et al. 2000) TEWL measures in preterm infants have also been shown to decrease relatively quickly during the first few days and then level off during the first month of life. (Agren, Sjörs et al. 1998; Kalia, Nonato et al. 1998) These differences in results could be explained by the differences in study design between our study and previous studies that were conducted in more well climate-controlled/consistent environments. TEWL in our study was measured in a community setting with consistently high humidity, therefore an infant's skin may not be undergoing the changes one would normally expect when adapting from a warm, humid environment in utero, to a cool, dry extrauterine environment.

Although the values of mean TEWL were similar at all visits for both treatment groups (apart from visit 3 in the preterm group), mean TEWL decreased at a faster rate in the sunflower oil group than in the mustard oil group with the largest difference in rate in the complete data set occurring from days 4 to 28, with an estimated 0.23 $g/m^2/hr$ per day faster decrease in the sunflower oil group. In addition, the preterm group had an estimated 0.47 $g/m^2/hr$ per day faster decrease in TEWL per day in the sunflower oil group between days 4 and 28. This could indicate that although the values of TEWL in this population are high, the skin barrier may be improving more rapidly in the sunflower oil group in preterm infants.

There was little evidence that skin condition scores were different between the two treatment groups. This is in contrast with a study done in Egypt, which resulted in a significant improvement in skin condition in a group of preterm infants massaged with sunflower oil. (Darmstadt, Badrawi et al. 2004) However, the Egypt study was conducted in a neonatal intensive care unit with a controlled climate, while this study was conducted in a community setting. In addition, that study was comparing the use of topical sunflower oil application with the use of minimal or no topical emollients, whereas in this study all participants were massaged with oil. All body regions in this study for both treatment groups showed increasing skin condition scores (worsening skin condition) until about two weeks of life followed by decreasing skin condition scores (improving skin condition) until day 28. This is in contrast to data from other studies that found rash only in a diapered region due to fecal contact and did not find rash in the chest region. (Visscher, Odio et al. 2009) More research should be done as to whether this is an effect of the oil (regardless of type) or whether it is a condition of the hot and humid environment (possibly causing infants to develop miliaria as a result of retention of sweat). (Foster, Hey et al. 1969; Zuniga and Nguyen 2013) This possible increase in rash and erythema due to climate could have nullified any improvement in skin condition from the different emollients.

Both treatment groups showed the same trend of decreasing stratum corneum protein concentration until around day 7, with a leveling off period, and then a slight increase from day 14 to 28, indicating that in both groups the skin barrier is improving (less stratum corneum protein is being removed and the barrier is more cohesive) during the first week of life, signifying the maturation of the stratum corneum. (Berthaud and Boncheva 2011; Myer and Maibach 2013) The slight increase in protein concentration during the second two weeks of life could indicate the stratum corneum is starting to regenerate, causing old skin cells to shed and the stratum corneum to be less cohesive. (Chiou and Blume-Peytavi 2004) Full term infants in the late neonatal period in the sunflower oil group were estimated to have stratum corneum protein concentrations 1.26 $\mu g/cm^2$ higher than in the mustard oil group. This may indicate a faster regeneration of the stratum corneum.

Although this study had many strengths, including the cluster-randomized design and the large sample size with an oversampling of preterm infants, there were some limitations. This study population has strong cultural practices of universal oil massage during the newborn period, which were not changed for this study. (Mullany, Darmstadt et al. 2005) Therefore, this study did not include a control group who did not receive full body oil massage or massage without oil and consequently was not able to compare the skin integrity measurements to infants who did not receive any emollient therapy or massage. In addition, the way in which newborn full body massage is practiced in Nepal, which is traditionally done in a very vigorous manner, was not changed and could potentially be harmful to the skin barrier. Vigorous rubbing could damage the stratum corneum, negating any possible beneficial effects of the emollients. (Chiou and Blume-Peytavi 2004)

In addition, due to the fact that measurements were made at homes in this rural community it was sometimes difficult to follow the recommended measurement protocols for the instruments (VapoMeters and skin pH meters). For example, the standard TEWL measurement protocol normally requires the skin to be bare and in a still position for up to 30 minutes prior to measuring so that it acclimatizes to the environment. (Aki Immonen, personal communication) This was not possible in our study, which may have resulted in higher measurements as infants usually had their skin covered before and between measurements, possibly increasing sweating. In addition, it is recommended that TEWL measurements be taken in an environment that is less than 50% relative humidity and between 20-22°C, which never occurred during our study period. (Rogeries and Group 2001) A similar, temperature controlled environment is recommended for skin pH measures. (Parra and Paye 2003) However, as infants from both oil groups were assessed in the same environment, this should not have impacted whether we could detect a difference between oil groups.

A mother's recalled date of last menstrual period (LMP) was relied on in order to determine gestational age, which may have resulted in inaccurate estimates of gestational age in our study. A study done in a tertiary-care hospital in Bangladesh found that LMP underestimated gestational age by one day compared with estimation from ultrasound. (Rosenberg, Ahmed et al. 2009) In addition, a study done at a hospital in Pakistan found that only 65% of estimated gestational age of reported LMP were within 7 days and only 82% were within 14 days when compared to gestational age estimates using ultrasound. (Jehan, Zaidi et al. 2010) Despite the problems of the uncertainty of LMP date, in this community setting in rural Nepal, the low-cost and simple method of reported LMP is the best option for estimating gestational age. Also, LMPs from our study were collected as soon as a pregnancy was identified (following-up women every 5 weeks), so recall bias would be minimized. In order to determine the accuracy of using recalled LMP to estimate gestational age in this population and to determine the direction of possible misclassification, research should be done on a small number of women comparing LMP estimates to estimation from ultrasound. Analyses should also be performed limiting our preterm group to those <34 weeks in order to determine if there are effects of the oils on skin integrity in infants who are more premature.

Further research should be conducted in this population investigating whether massage with emollients has any effect on skin barrier function when compared to massage without emollients and to no massage. It is also important to assess if differences exist in these skin integrity measurements in infants who are massaged differently (i.e. vigorous versus gentle massage, or massage with moderate pressure). Both changing the way in which an infant is massaged and whether an infant is massaged would be very difficult in this community setting in rural Nepal, as neonatal oil massage is a nearly universal cultural practice. (Mullany, Darmstadt et al. 2005) However, a smaller group of neonates in an urban area may be more willing to accept these behavioral change interventions. Another possibility in this rural community is to enroll older infants past the age when it is considered a cultural necessity to massage the infants, in order to get control groups of massage without oil or no massage. However, in this type of population, the maturation of the skin barrier could not be measured.

Additional research should also be conducted on other possible biological mechanisms, which may be affected by neonatal full body oil massage with mustard oil or sunflower oil. These include differences of the effects of the different oils on bacterial colonization of the skin, differences in immune status and function, and differences in nutritional status. In addition, risk factors for skin integrity, bacterial colonization, immune function, and nutritional status should be examined in this population in order to better target groups of infants who may be at higher risk of poor skin integrity and function, poor immune function, poor nutrition, or colonization of certain bacteria. In addition, whether there are any correlations between the different skin integrity measurements (TEWL, skin pH, stratum corneum protein concentration, and skin condition) should be explored as well as any relationships between different biological mechanisms (skin integrity, bacterial colonization, nutritional status, and immune function). Relationships between morbidity and skin integrity should also be examined.

These results indicate a possible faster rate of decrease in skin pH and TEWL in infants when massaged with sunflower oil versus mustard seed oil, which could be indicative of improved skin barrier function in infants massaged with sunflower oil. If there are improved health outcomes in neonates massaged with sunflower oil, improved skin barrier function could be one of the reasons. These results may also be applicable to northern India, Pakistan, and northwestern Bangladesh, as well as the Terai region in southern Nepal, as they share cultural, social, and economic characteristics. Benefits of topical emollient therapy should also be explored in other low-resource settings. It is important to have a better of understanding of the underlying mechanisms of how and why emollient therapy can improve neonatal health outcomes in low-resource settings, because the optimization of health benefits of oils depends upon the understanding of which biological mechanisms are impacted by the use of different oils.

Chapter 5 Tables and Figures

Table 5-1: Scheduling of Visits and Associated Data Items Collected

Measure	# of Infants	Visit 1	Visit 3	Visit 7	Visit 14	Visit 28
Skin Condition Score	1000	X	X	X	X	X
TEWL	1000	X	X	X	X	X
Skin pH	1000	X	X	X	X	X
Skin Discs to Measure Protein Concentration	1000	X		X	X	X

Table 5-2: Baseline Maternal, Socioeconomic, Household, and Newborn Care Characteristics by Intervention Group

	Oil Randomization Group			
	Mustard Oil		Sunflower Oil	
	N	(%)	N	(%)
Number of Clusters	56		58	
Total number of infants	324		308	
Ethnic Group				
Pahadi	15	4.8	29	9.5
Madeshi	300	95.2	277	90.5
Maternal literacy				
Not literate	229	71.3	220	71.2
Literate	92	28.7	89	28.8
Paternal literacy				
Not literate	153	47.7	141	45.6
Literate	168	52.3	168	54.4
Maternal Education				
None	229	71.3	221	71.5
1-5 yrs	30	9.4	29	9.4
6-10 yrs	47	14.6	47	15.2
>10 yrs	15	4.7	12	3.9
Paternal Education				
None	147	45.8	142	46.0
1-5 yrs	48	15.0	48	15.5
6-10 yrs	100	31.2	105	34.0
>10 yrs	26	8.1	14	4.5
Household Assets[§]				
0 or 1 asset(s)	22	7.0	22	7.2
2-5 assets	135	42.9	143	46.7
6-10 assets	150	47.6	133	43.5
>10 assets	8	2.5	8	2.6
Electricity				
No	112	35.6	140	45.8
Yes	203	64.4	166	54.3
Maternal Age				
<18 yrs	43	13.4	41	13.3
18-24 yrs	177	55.1	180	58.3
25-29 yrs	72	22.4	63	20.4
30-34 yrs	23	7.2	13	4.2
>=35 yrs	6	1.9	12	3.9

Table 5-2: Baseline Maternal, Socioeconomic, Household, and Newborn Care Characteristics by Intervention Group (continued)

	Oil Randomization Group			
	Mustard Oil		Sunflower Oil	
	N	(%)	N	(%)
Gravidity				
None	83	25.9	83	26.9
1-2	143	44.6	152	49.2
3-4	85	26.5	70	22.7
>5	10	3.1	4	1.3
Parity				
None	88	27.4	91	29.5
1-2	135	42.1	136	44.0
3-4	82	25.6	73	23.6
>5	16	5.0	9	2.9
Antenatal Care Visits				
No ANC	90	28.0	92	29.8
1-2 ANC visits	122	38.0	109	35.3
3-4 ANC visits	94	29.3	96	31.1
>=5 ANC visits	15	4.7	12	3.9
Location of Delivery				
Home	163	50.8	160	51.8
Maiti	57	17.8	52	16.8
HP/Clinic	54	16.8	58	18.8
Hospital	36	11.2	32	10.4
On way to facility	11	3.4	7	2.3
Length of Labor				
<5 hrs	147	45.8	149	48.4
5-14 hrs	144	44.7	118	38.3
15-19 hrs	11	3.4	16	5.2
20-24 hrs	10	3.1	14	4.6
>24 hrs	9	2.8	11	3.6
Complications during Delivery				
No	267	83.2	246	80.1
Yes	54	16.8	61	19.9
Delivery Assistant				
No one/Family/Neighbors	81	25.3	78	25.2
TBA/Chamain	57	17.8	61	19.7
CHV/VHW/MCH Worker	14	4.4	8	2.6
ANM/HA/CMA/Staff Nurse	84	26.3	86	27.8
Local doctor	79	24.7	73	23.6
MBBS doctor	5	1.6	3	1.0

Table 5-2: Baseline Maternal, Socioeconomic, Household, and Newborn Care Characteristics by Intervention Group (continued)

	Oil Randomization Group			
	Mustard Oil		Sunflower Oil	
	N	(%)	N	(%)
Sex				
Male	169	51.8	160	51.4
Female	157	48.2	151	48.6
Gestational Age				
<32 wks	20	6.3	17	5.6
32-36 wks	124	39.1	104	34.3
37-41 wks	148	46.7	157	51.8
>=42 wks	25	7.9	25	8.3
Birthweight				
<1500g	4	1.3	8	2.6
1500-2499g	104	32.6	115	37.7
>2500g	211	66.1	182	59.7
SGA Status				
AGA	185	58.0	172	56.4
SGA 3-10%	62	19.4	57	18.7
SGA 3%	72	22.6	76	25.0
Breastfed since birth at first visit				
No	54	16.6	42	13.5
Yes	272	83.4	269	86.5
Hours before breastfeeding initiation				
<1 hr	87	32.3	74	27.8
1-2 hrs	154	57.2	151	56.8
3-4 hrs	15	5.6	20	7.5
>=5 hrs	13	4.8	21	7.9
Infants received colostrum				
No	30	11	27	10
Yes	241	88.6	242	90
Gestational Age (wks) (mean, SD)	37.5	(3.7)	37.9	(3.5)
Birthweight (g) (mean, SD)	2639.4	(465.6)	2582.8	(501.3)

§: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table 5-3: Intervention Coverage by Intervention Group

	Mustard Oil		Sunflower Oil	
	N	%	N	%
# of follow-up visits* (mean, SD, range)	4.69 (±0.81) 1-5		4.77 (±0.66) 1-5	
# of times massaged in first week (mean, SD, range)	32.34 (±10.78) 1-83		29.79 (±9.36) 3-63	
Avg # of times massaged per day in first week (mean, SD, range)	4.70 (±1.47) 1-11.9		4.31 (±1.27) 1.5-9	
Massaged since last visit§				
Visit 1	323	99.7	308	100
Visit 3	315	100	302	99.7
Visit 7	311	99.7	301	98.7
Visit 14	305	98.7	290	97.0
Visit 28	269	99.6	259	98.9
Massaged with NNIPS oil during last massage				
Visit 1	298	92.3	254	82.5
Visit 3	315	100	283	93.7
Visit 7	299	96.1	272	90.3
Visit 14	294	96.4	269	92.8
Visit 28	260	96.7	229	88.4
Time between visit and last massage				
<30 min	302	20.0	313	21.7
30-59 min	267	17.7	237	16.4
60-119 min	355	23.5	326	22.6
120-179 min	205	13.6	192	13.3
>180 min	382	25.3	375	26.0

*Max number of household visits is 5; § At visit 1 this is massaged since birth

Table 5-4: Descriptive Characteristics of TEWL by Visit and Intervention Group

Visit Number	Mustard Oil				Sunflower Oil			
	N	n	Mean ($g/m^2/hr$)	SD ($g/m^2/hr$)	N	n	Mean ($g/m^2/hr$)	SD ($g/m^2/hr$)
Complete Data Set								
1	322	56	34.34	22.47	307	57	35.34	23.34
3	314	56	36.21	24.25	302	57	39.36	24.45
7	310	56	37.92	22.96	301	57	40.53	24.95
14	305	56	41.70	24.69	288	57	41.32	23.85
28	266	56	44.79	23.88	254	57	41.74	22.50
Full Term								
1	173	51	36.13	22.38	184	52	35.19	22.92
3	168	51	38.10	24.46	179	52	37.93	23.45
7	165	51	38.16	23.37	179	51	40.98	24.65
14	164	51	42.14	24.68	178	52	43.14	23.94
28	143	50	45.43	23.25	150	51	44.26	22.78
Preterm								
1	143	53	31.60	21.64	120	47	35.52	24.19
3	141	53	33.81	23.85	120	47	41.49	25.89
7	138	53	37.36	22.36	119	47	40.36	25.53
14	136	53	40.11	24.00	108	47	38.27	23.49
28	119	51	43.63	24.19	102	45	38.09	21.88

N=number of observations, n=number of clusters, SD=standard deviation

Table 5-5: Regression Results of Intervention Group on TEWL

	N	n	Coefficient ($g/m^2/hr$)	SE ($g/m^2/hr$)	95% CI ($g/m^2/hr$)
Complete Data Set					
Visit 1	629	113	0.70	2.06	-3.32-4.72
Visit 3	615	113	3.29	2.23	-1.07-7.66
Visit 7	608	113	2.59	2.10	-1.52-6.71
Visit 14	592	113	-0.35	2.02	-4.31-3.60
Visit 28	519	113	-3.05	2.03	-7.04-0.94
Complete Neonatal Period	631	113	0.99	1.67	-2.26-4.24
Early Neonatal Period	631	113	1.48	1.89	-2.23-5.18
Late Neonatal Period	610	113	0.64	1.77	-2.83-4.11
Full Term					
Visit 1	357	103	-1.33	2.56	-6.35-3.69
Visit 3	347	103	-0.10	2.66	-5.32-5.12
Visit 7	344	102	2.84	2.66	-2.36-8.04
Visit 14	342	103	1.00	2.62	-4.14-6.14
Visit 28	293	101	-1.17	2.68	-6.42-4.08
Complete Neonatal Period	358	104	0.61	1.92	-3.14-4.36
Early Neonatal Period	358	104	-0.65	2.22	-4.99-3.70
Late Neonatal Period	347	103	2.19	2.17	-2.07-6.45
Preterm					
Visit 1	264	100	2.80	3.19	-3.45-9.05
Visit 3	261	100	7.27*	3.38	0.65-13.89
Visit 7	257	100	2.56	3.15	-3.61-8.73
Visit 14	244	100	-1.84	3.05	-7.82-4.14
Visit 28	221	96	-5.54	3.11	-11.63-0.56
Complete Neonatal Period	265	100	1.35	2.41	-3.39-6.09
Early Neonatal Period	265	100	3.38	2.99	-2.48-9.24
Late Neonatal Period	256	100	-1.07	2.60	-6.16-4.02

N=number of observations for visits and number of infants for neonatal periods, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE=standard errors, 95% CI=95% confidence interval, *=p<0.05

Table 5-6: Descriptive Characteristics of Skin pH by Visit and Intervention Group

Visit Number	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Complete Data Set								
1	322	56	5.95	0.51	308	57	6.12	0.53
3	314	56	5.55	0.50	302	57	5.63	0.48
7	310	56	5.15	0.55	304	57	5.22	0.53
14	305	56	5.07	0.56	297	57	5.07	0.55
28	266	56	4.83	0.60	255	57	4.82	0.53
Full Term								
1	174	52	5.98	0.51	184	52	6.15	0.56
3	168	51	5.55	0.50	179	52	5.60	0.49
7	167	51	5.14	0.56	182	52	5.20	0.54
14	165	51	5.07	0.57	180	52	5.05	0.58
28	142	49	4.83	0.58	150	52	4.79	0.52
Preterm								
1	143	53	5.93	0.51	121	47	6.09	0.48
3	142	53	5.56	0.50	120	42	5.67	0.47
7	139	52	5.17	0.54	119	47	5.24	0.51
14	136	53	5.08	0.55	115	47	5.11	0.49
28	121	51	4.82	0.62	103	45	4.86	0.54

N=number of observations, n=number of clusters, SD=standard deviation

Table 5-7: Regression Results of Intervention Group on Skin pH

	N	n	Coefficient	SE	95% CI
Complete Data Set					
Visit 1	630	113	0.16**	0.05	0.07-0.25
Visit 3	616	113	0.07	0.05	-0.02-0.16
Visit 7	614	113	0.07	0.05	-0.03-0.17
Visit 14	602	113	0.01	0.05	-0.09-0.11
Visit 28	521	113	-0.01	0.05	-0.11-0.08
Complete Neonatal Period	632	112	0.06	0.04	-0.02-0.13
Early Neonatal Period	632	113	0.12**	0.04	0.04-0.20
Late Neonatal Period	612	113	0.05	0.05	-0.04-0.14
Full Term					
Visit 1	358	104	0.17**	0.06	0.06-0.28
Visit 3	347	103	0.05	0.05	-0.06-0.15
Visit 7	349	103	0.07	0.06	-0.06-0.19
Visit 14	345	103	-0.02	0.06	-0.14-0.10
Visit 28	292	100	-0.05	0.07	-0.18-0.08
Complete Neonatal Period	359	104	0.04	0.04	-0.04-0.12
Early Neonatal Period	359	104	0.12*	0.05	0.02-0.21
Late Neonatal Period	348	103	0.04	0.05	-0.06-0.14
Preterm					
Visit 1	264	100	0.16*	0.06	0.03-0.28
Visit 3	262	100	0.11	0.07	-0.02-0.24
Visit 7	258	99	0.08	0.07	-0.06-0.22
Visit 14	251	100	0.04	0.07	-0.11-0.18
Visit 28	224	96	0.04	0.08	-0.11-0.19
Complete Neonatal Period	265	100	0.09	0.05	-0.01-0.18
Early Neonatal Period	265	100	0.13*	0.05	0.03-0.23
Late Neonatal Period	257	100	0.07	0.07	-0.06-0.20

N=number of observations for visits and number of infants for neonatal periods, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE=standard errors, 95% CI=95% confidence interval, *=p<0.05, **=p<0.01

Table 5-8: Cox Hazard Regression Results of Intervention Group on Skin pH Adjusted for Skin pH Measure at Visit 1

	N	n	Hazard Ratio	SE	95% CI
Complete Data Set	629	113	1.13	0.10	0.95-1.34
Full Term	357	104	1.04	0.13	0.82-1.33
Preterm	264	100	1.23	0.17	0.94-1.62

N=number of observations, n=number of clusters, SE= standard errors, 95% CI=95% confidence interval

Table 5-9: Descriptive Characteristics of Stratum Corneum Protein Concentration by Visit and Intervention Group

Visit Number	Mustard Oil				Sunflower Oil			
	N	n	Mean ($\mu g/cm^2$)	SD ($\mu g/cm^2$)	N	n	Mean ($\mu g/cm^2$)	SD ($\mu g/cm^2$)
Complete Data Set								
1	264	54	16.07	8.14	247	55	16.75	7.61
7	259	54	12.45	6.26	248	56	13.40	6.52
14	253	54	12.89	6.12	247	56	13.20	6.52
28	224	55	13.68	6.26	213	55	14.36	5.86
Full Term								
1	141	49	15.82	8.47	144	49	16.65	8.12
7	137	47	11.70	6.26	148	51	13.54	6.40
14	136	46	12.60	6.26	148	52	13.28	6.55
28	121	45	13.26	6.00	123	49	13.94	5.92
Preterm								
1	119	52	16.26	7.84	100	43	16.90	6.85
7	118	51	13.17	6.15	98	42	13.27	6.77
14	114	50	13.13	4.84	97	42	13.21	6.51
28	101	48	14.25	6.58	88	39	14.93	5.83

N=number of observations, n=number of clusters, SD=standard deviation

Table 5-10: Regression Results of Intervention Group on Protein Concentration

	N	n	Coefficient (μg/cm²)	SE (μg/cm²)	95% CI (μg/cm²)
Complete Data Set					
Visit 1	511	109	0.64	0.73	-0.79-2.07
Visit 3	507	110	0.97	0.65	-0.30-2.25
Visit 7	500	110	0.31	0.56	-0.79-1.42
Visit 14	437	110	0.69	0.59	-0.46-1.83
Visit 28	511	109	0.64	0.73	-0.79-2.07
Complete Neonatal Period	572	111	0.61	0.37	-0.10-1.33
Early Neonatal Period	522	110	0.84	0.59	-0.33-2.00
Late Neonatal Period	533	110	0.55	0.43	-0.29-1.40
Full Term					
Visit 1	285	98	0.75	1.02	-1.25-2.74
Visit 3	285	98	1.84*	0.75	0.37-3.31
Visit 7	284	98	0.68	0.76	-0.81-2.17
Visit 14	244	94	0.68	0.76	-0.81-2.17
Visit 28	285	98	0.75	1.02	-1.25-2.74
Complete Neonatal Period	321	101	0.97*	0.46	0.07-1.87
Early Neonatal Period	291	99	0.79	0.83	-0.84-2.42
Late Neonatal Period	299	99	1.26*	0.57	0.15-2.38
Preterm					
Visit 1	219	95	0.63	1.08	-1.48-2.74
Visit 3	216	93	0.27	1.03	-1.74-2.29
Visit 7	211	92	0.08	0.85	-1.58-1.74
Visit 14	189	87	0.69	0.91	-1.09-2.46
Visit 28	219	95	0.63	1.08	-1.48-2.74
Complete Neonatal Period	244	98	0.35	0.54	-0.72-1.42
Early Neonatal Period	224	96	1.08	0.82	-0.52-2.68
Late Neonatal Period	228	94	-0.22	0.67	-1.54-1.10

N=number of observations for visits and number of infants for neonatal periods, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE=standard errors, 95% CI=95% confidence interval, *=p<0.05

Table 5-11: Descriptive Characteristics of Chest Skin Condition Score by Visit and Intervention Group-Complete Data

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	324	56	0.51	0.65	308	57	0.55	0.69
Rash	324	56	0.14	0.40	308	57	0.16	0.44
Total	324	56	0.64	0.85	308	57	0.70	0.93
Visit 3								
Erythema	315	56	0.84	0.65	303	57	0.82	0.66
Rash	315	56	0.75	0.76	303	57	0.64	0.77
Total	315	56	1.59	1.24	303	57	1.47	1.11
Visit 7								
Erythema	312	56	0.78	0.62	305	57	0.83	0.65
Rash	312	56	0.79	0.79	305	57	0.81	0.82
Total	312	56	1.57	1.22	305	57	1.64	1.30
Visit 14								
Erythema	309	56	0.83	0.63	299	57	0.83	0.65
Rash	309	56	1.04	0.77	299	57	0.99	0.82
Total	309	56	1.88	1.28	299	57	1.81	1.35
Visit 28								
Erythema	270	56	0.55	0.58	262	57	0.52	0.59
Rash	270	56	0.70	0.72	262	57	0.66	0.73
Total	270	56	1.25	1.17	262	57	1.19	1.22

N=number of observations, n=number of clusters, SD=standard deviation

Table 5-12: Descriptive Characteristics of Chest Skin Condition Score by Visit and Intervention Group-Full Term

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	175	52	0.51	0.63	184	52	0.48	0.63
Rash	175	52	0.13	0.40	184	52	0.20	0.49
Total	175	52	0.64	0.82	184	52	0.68	0.93
Visit 3								
Erythema	169	51	0.84	0.63	180	52	0.81	0.66
Rash	169	51	0.72	0.73	180	52	0.64	0.76
Total	169	51	1.56	1.19	180	52	1.44	1.20
Visit 7								
Erythema	168	51	0.76	0.60	183	52	0.81	0.65
Rash	168	51	0.82	0.80	183	52	0.90	0.82
Total	168	51	1.58	1.21	183	52	1.71	1.32
Visit 14								
Erythema	167	51	0.91	0.62	181	52	0.86	0.65
Rash	167	51	1.14	0.79	181	52	1.10	0.80
Total	167	51	2.05	1.27	181	52	1.96	1.36
Visit 28								
Erythema	144	50	0.54	0.58	155	51	0.51	0.58
Rash	144	50	0.73	0.72	155	51	0.71	0.73
Total	144	50	1.27	1.19	155	51	1.22	1.22

N=number of observations, n=number of clusters, SD=standard deviation

Table 5-13: Descriptive Characteristics of Chest Skin Condition Score by Visit and Intervention Group-Preterm

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	144	53	0.51	0.67	121	47	0.66	0.78
Rash	144	53	0.15	0.41	121	47	0.10	0.35
Total	144	53	0.65	0.90	121	47	0.76	0.93
Visit 3								
Erythema	142	53	0.84	0.68	120	47	0.85	0.67
Rash	142	53	0.80	0.79	120	47	0.66	0.78
Total	142	53	1.64	1.30	120	47	1.51	1.28
Visit 7								
Erythema	140	53	0.82	0.64	119	47	0.87	0.65
Rash	140	53	0.76	0.80	119	47	0.70	0.81
Total	140	53	1.58	1.23	119	47	1.58	1.26
Visit 14								
Erythema	138	53	0.75	0.63	116	47	0.78	0.64
Rash	138	53	0.92	0.75	116	47	0.83	0.83
Total	138	53	1.68	1.26	116	47	1.61	1.30
Visit 28								
Erythema	123	51	0.56	0.57	105	45	0.54	0.61
Rash	123	51	0.67	0.72	105	45	0.60	0.72
Total	123	51	1.24	1.15	105	45	1.14	1.23

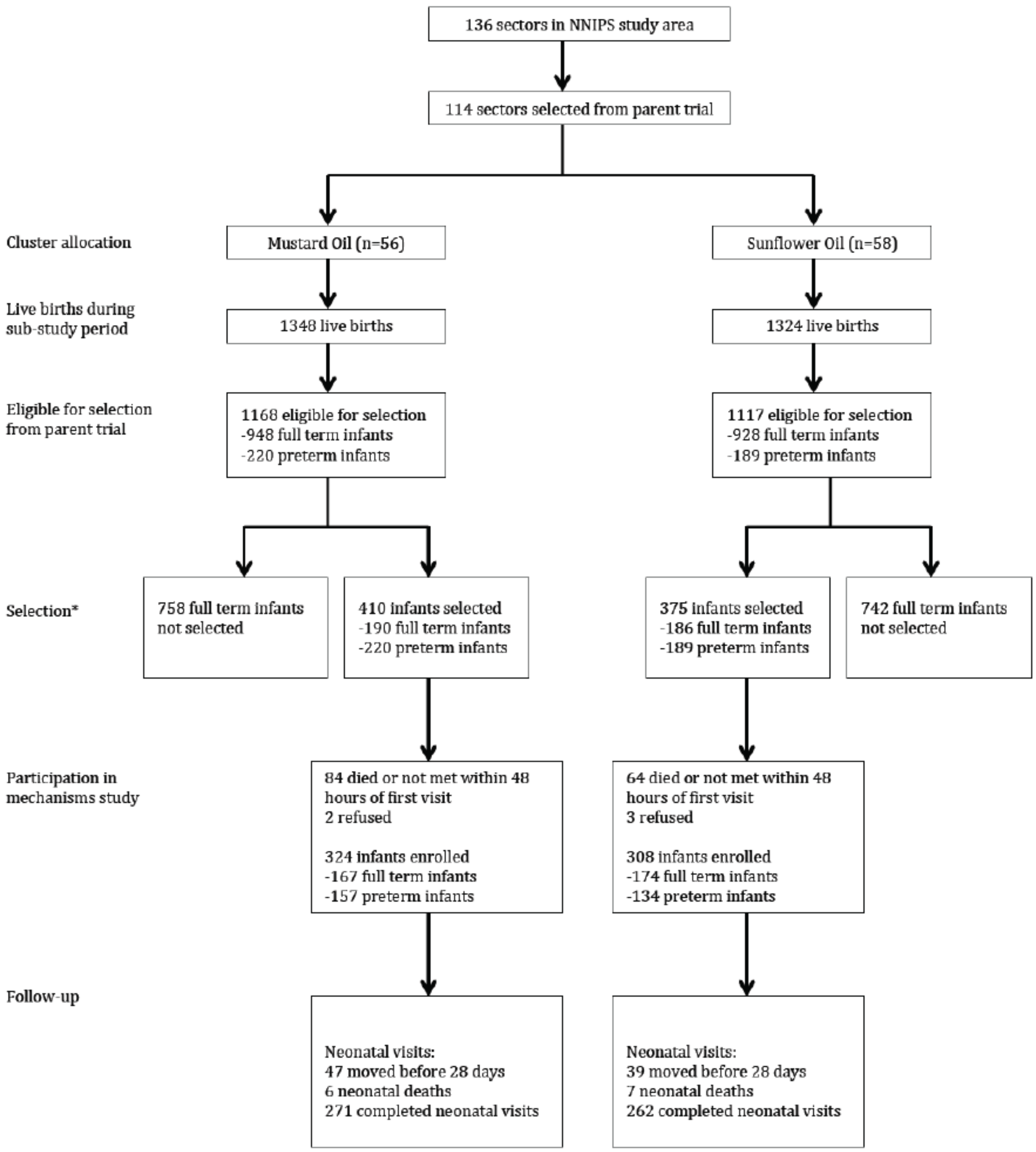
N=number of observations, n=number of clusters, SD=standard deviation

Table 5-14: Regression Results of Intervention Group on Chest Skin Condition Total Score

	N	n	Coefficient	SE	95% CI
Complete Data Set					
Visit 1	632	113	0.06	0.07	-0.08-0.20
Visit 3	618	113	-0.13	0.10	-0.32-0.07
Visit 7	617	113	0.07	0.10	-0.14-0.27
Visit 14	608	113	-0.06	0.11	-0.27-0.14
Visit 28	532	113	-0.07	0.11	-0.29-0.16
Complete Neonatal Period	632	113	-0.03	0.06	-0.14-0.10
Early Neonatal Period	632	113	-0.03	0.07	-0.16-0.11
Late Neonatal Period	632	113	-0.004	0.09	-0.18-0.17
Full Term					
Visit 1	359	104	0.03	0.11	-0.17-0.24
Visit 3	349	103	-0.11	0.13	-0.36-0.13
Visit 7	351	103	0.13	0.14	-0.13-0.40
Visit 14	348	103	-0.11	0.15	-0.41-0.19
Visit 28	299	101	-0.05	0.13	-0.33-0.22
Complete Neonatal Period	359	104	-0.01	0.08	-0.16-0.15
Early Neonatal Period	359	104	0.02	0.09	-0.16-0.20
Late Neonatal Period	359	104	-0.03	0.12	-0.26-0.21
Preterm					
Visit 1	265	100	0.10	0.11	-0.12-0.32
Visit 3	262	100	-0.13	0.16	-0.45-0.18
Visit 7	259	100	-0.01	0.16	-0.32-0.30
Visit 14	254	100	-0.07	0.17	-0.39-0.25
Visit 28	228	96	-0.10	0.17	-0.43-0.23
Complete Neonatal Period	265	100	-0.04	0.09	-0.23-0.14
Early Neonatal Period	265	100	-0.06	0.10	-0.26-0.15
Late Neonatal Period	257	100	0.002	0.13	-0.26-0.26

N=number of observations for visits and number of infants for neonatal periods, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE=standard errors, 95% CI=95% confidence interval

Figure 5-1: Study Flow Diagram



*20% of full term neonates were randomly selected for inclusion and all preterm neonates were selected for inclusion in the sub-study.

Figure 5-2: TEWL by Infant's Age-Complete Data

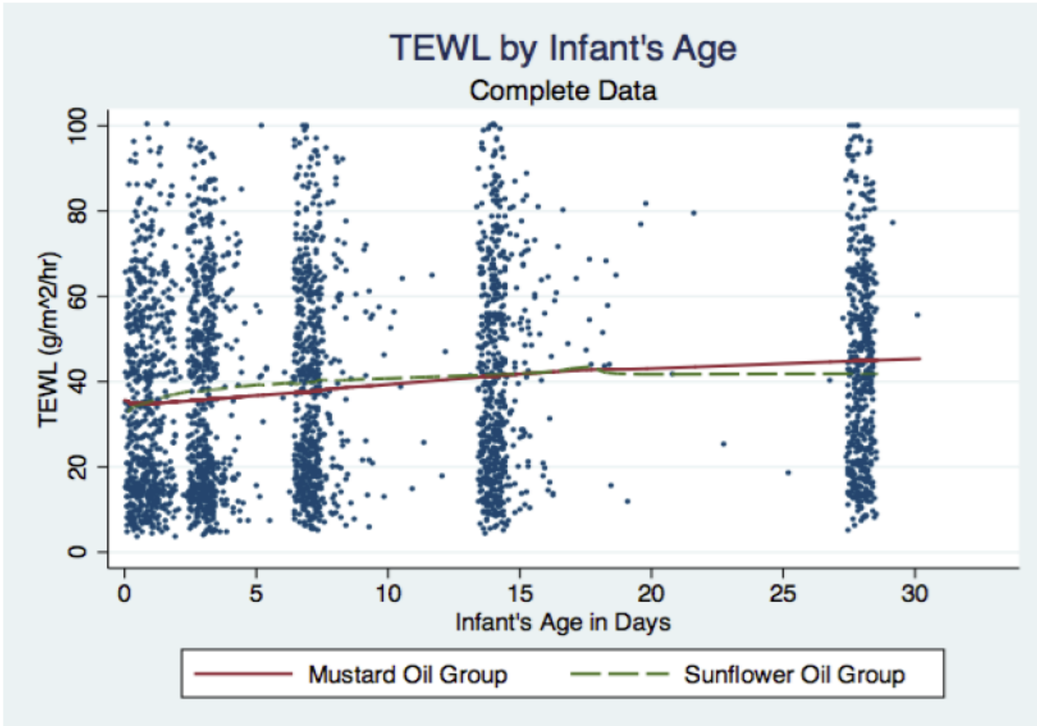


Figure 5-3: TEWL by Infant's Age-Preterm

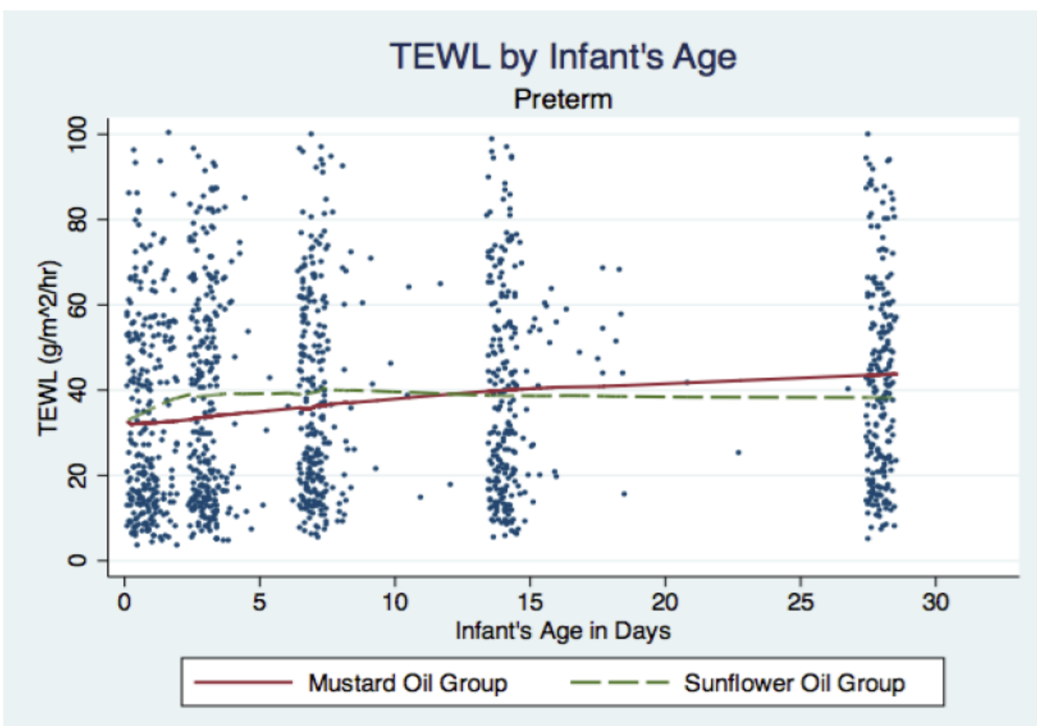


Figure 5-4: Skin pH by Infant's Age-Complete Data

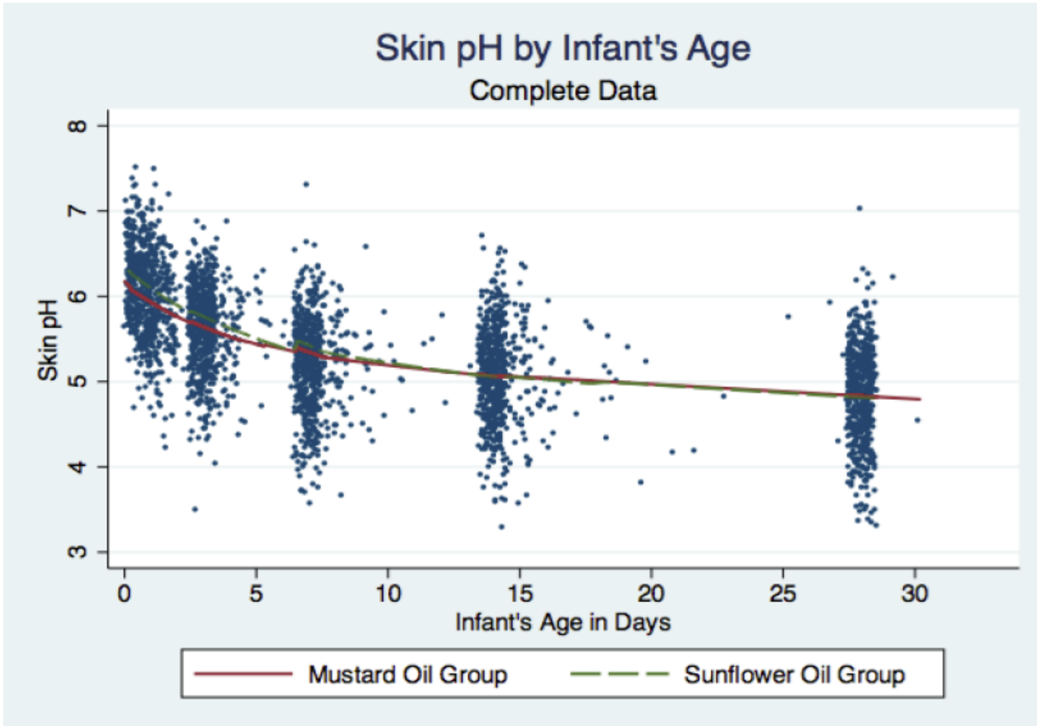


Figure 5-5: Skin pH by Infant's Age-Preterm

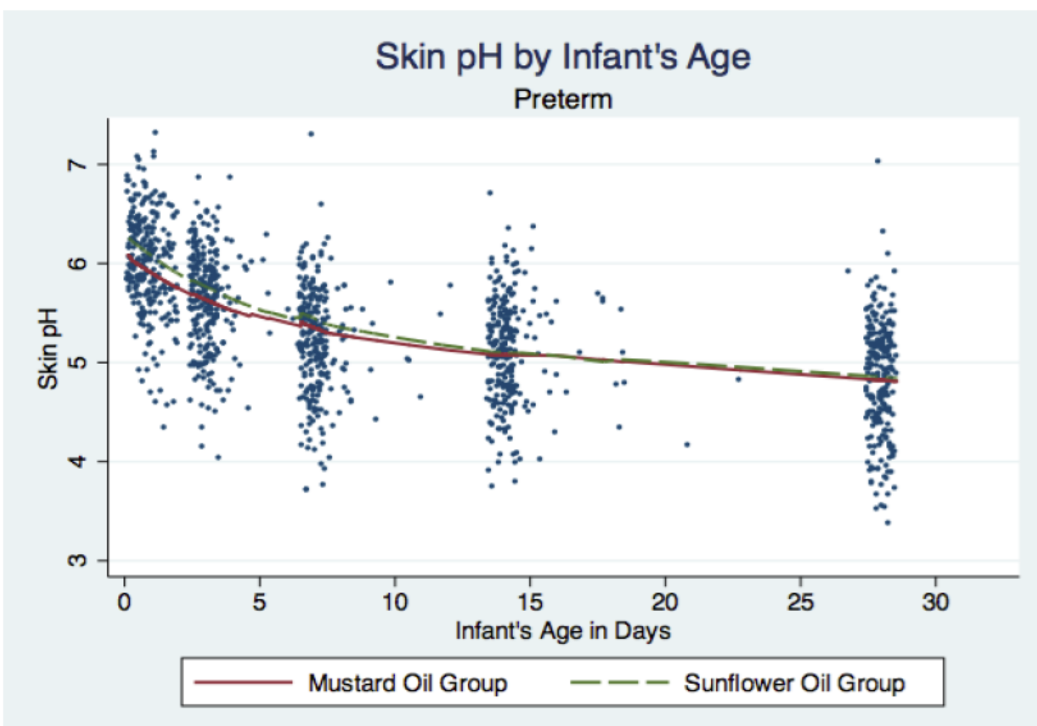


Figure 5-6: Skin pH Cox Proportional Hazards Regression Survival Curves-Complete Data

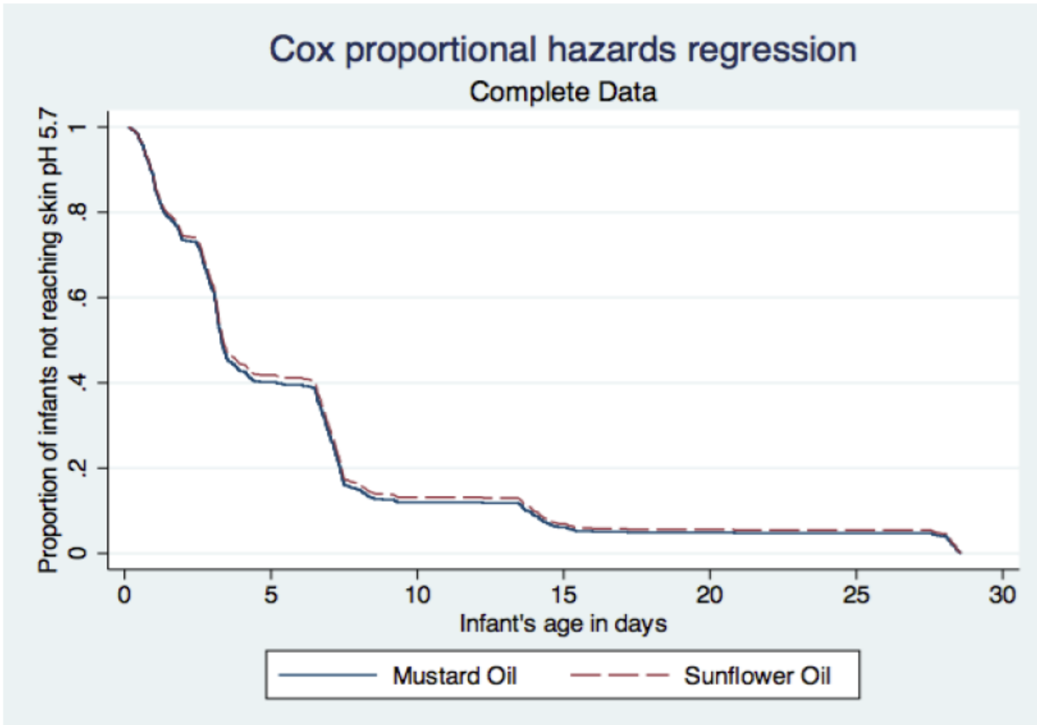


Figure 5-7: Skin pH Cox Proportional Hazards Regression Survival Curves-Preterm

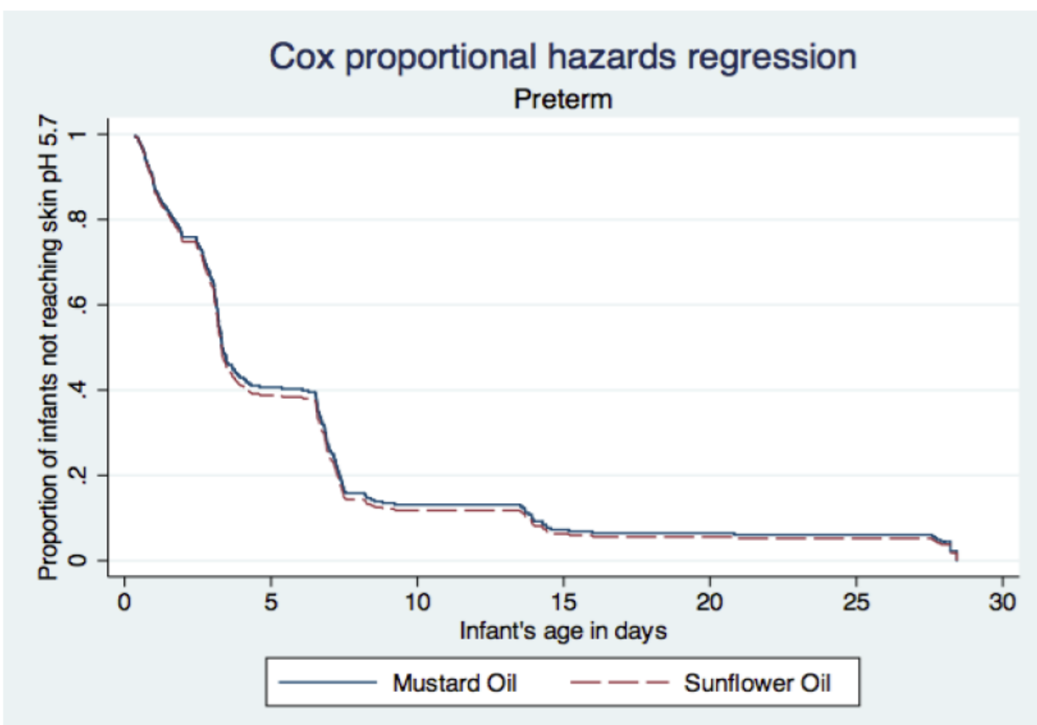


Figure 5-8: Stratum Corneum Protein Concentration by Infant's Age-Complete Data

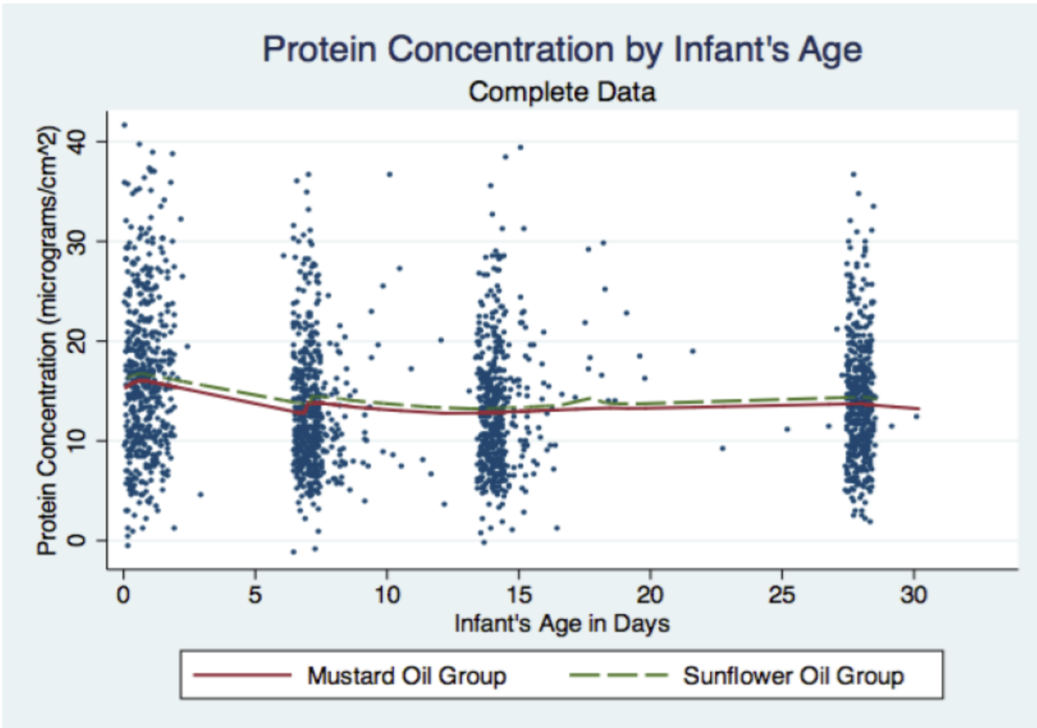


Figure 5-9: Stratum Corneum Protein Concentration by Infant's Age-Preterm

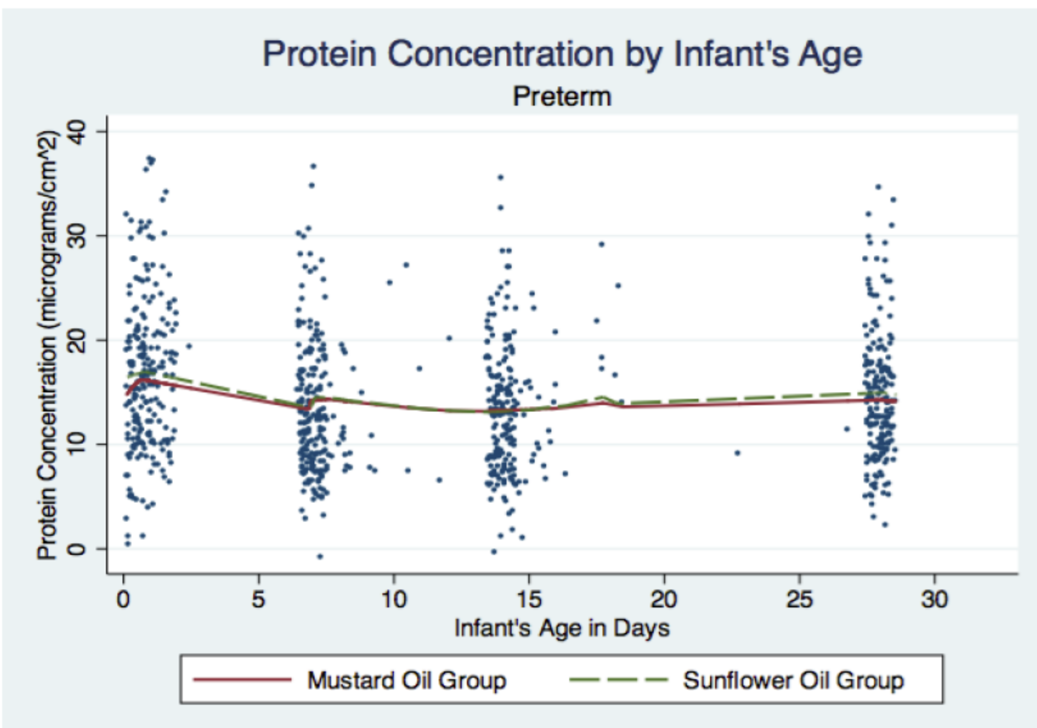


Figure 5-10: Chest Skin Condition Total Score by Infant's Age-Complete Data

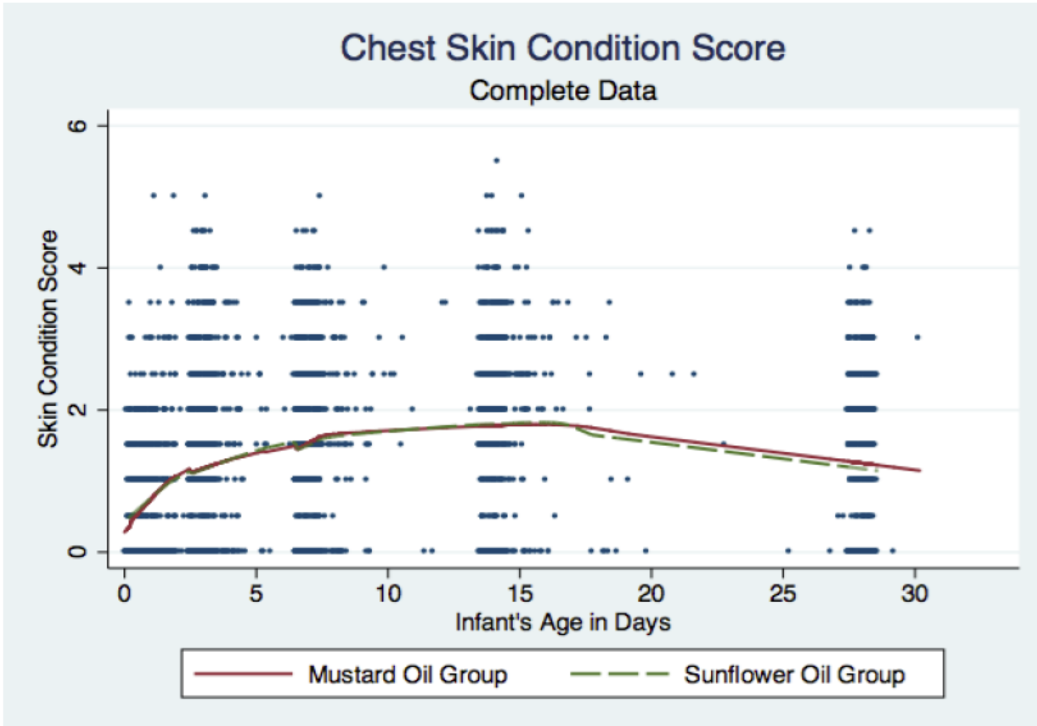


Figure 5-11: Chest Skin Condition Total Score by Infant's Age-Preterm

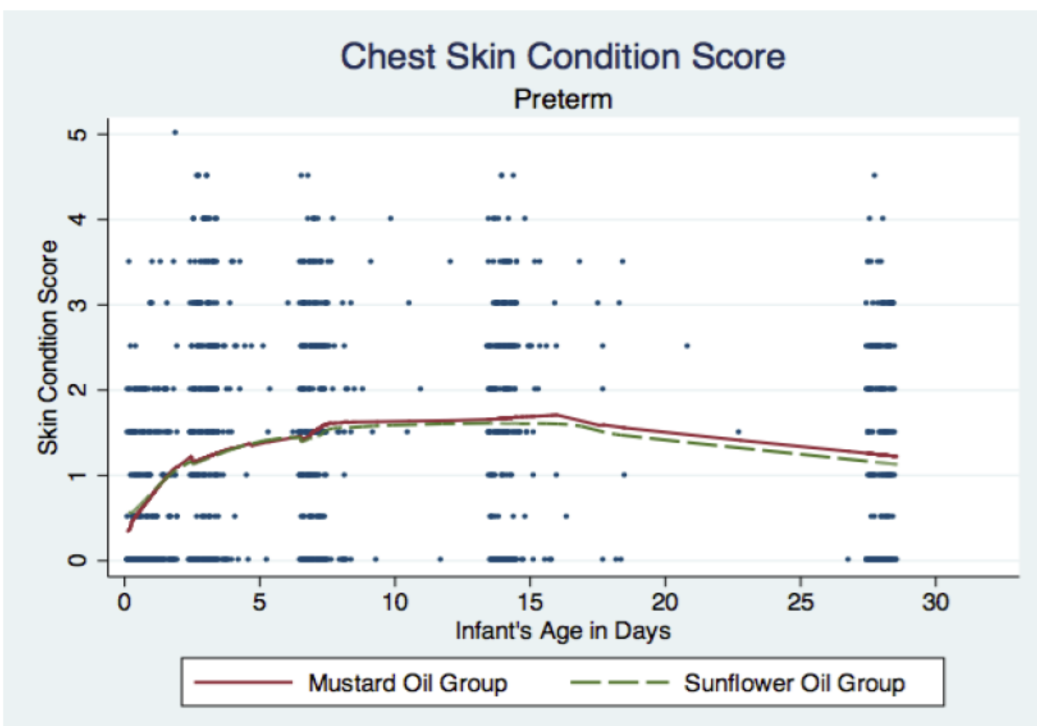
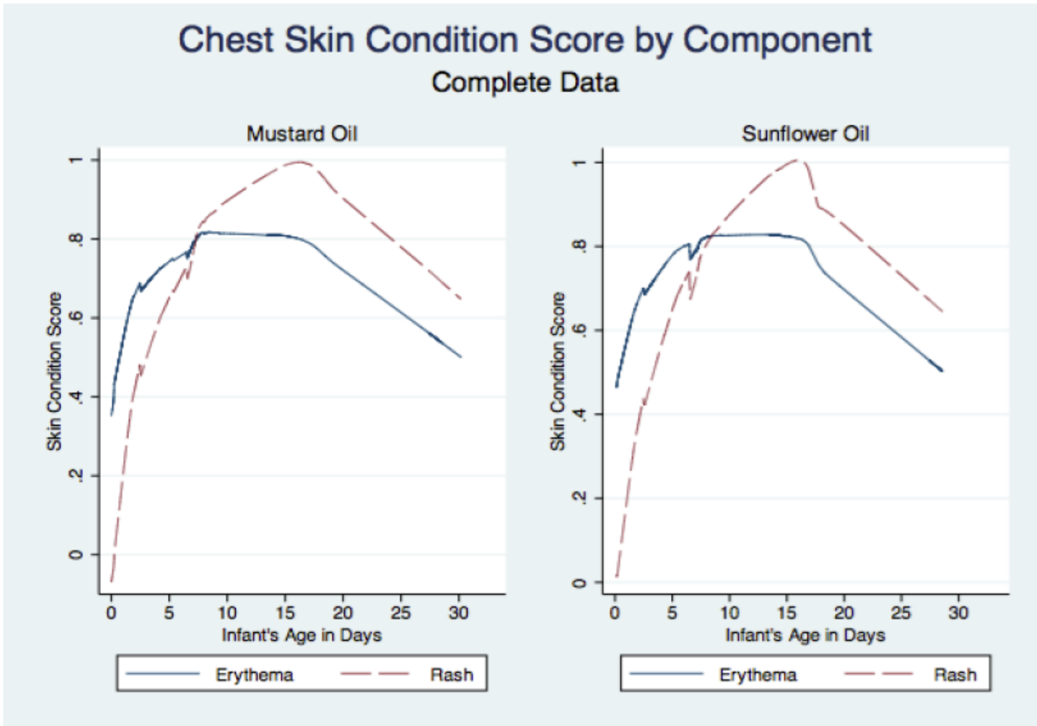


Figure 5-12: Chest Skin Condition Score by Component and Intervention Group-Complete Data



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Chapter 6 Effect of Oil Group on Nutritional Status

Background

Globally, 3.1 million neonatal (≤28 days) deaths occur each year, with 98% of these deaths occurring in developing countries. (Black, Cousens et al. 2010; Lawn, Kerber et al. 2010; Liu, Johnson et al. 2012) This accounts for >40% of all deaths in children under-five years, a proportion that has been increasing in recent years as gains in post-natal survival outpace those in the neonatal period. (Lawn, Cousens et al. 2005; Oestergaard, Inoue et al. 2011; Liu, Johnson et al. 2012) The top three causes of death in these infants are complications from preterm birth (35%), infections (including sepsis, pneumonia, diarrhea, meningitis, and tetanus) (27%), and intrapartum related neonatal deaths, such as birth asphyxia (23%). (Liu, Johnson et al. 2012) Preterm complications also contribute to other causes of death, resulting in half of all neonatal deaths globally. (Belizan, McClure et al. 2012) In Southeast Asia, 52% of child deaths occur during the neonatal period and in Nepal it is estimated that in 2010, 58% of under-five deaths occurred during this period. (Liu, Johnson et al. 2012)

The practice of neonatal oil massage using locally available vegetable oils is one intervention being explored to reduce neonatal mortality, which is practiced almost universally throughout Southeast Asia. (Darmstadt and Saha 2002; Mullany, Darmstadt et al. 2005) Although less widespread, oil massage is also practiced in many communities in Africa, and could be acceptable as an intervention to improve neonatal mortality and morbidity. (Iweze 1983; Niang 2004; Darmstadt, Hussein et al. 2007; Mrisho, Schellenberg et al. 2008; Waiswa, Nyanzi et al. 2010; Duffy, Ferguson et al. 2012)

Studies have shown that massage with oil is more beneficial than massage alone. Compared with non-massaged infants, those massaged with Johnson and Johnson baby oil demonstrated fewer stress behaviors, lower salivary cortisol levels, and increased vagal activity. (Field, Schanberg et al. 1996) A study in Egypt of preterm infants <34 weeks comparing the application of sunflower oil to controls showed a significant decrease in the incidence of nosocomial infections and an improvement in skin condition in infants receiving sunflower oil. (Darmstadt, Badrawi et al. 2004) A larger, similarly designed study of preterm infants in Bangladesh comparing infants randomized to massage with sunflower oil, Aquaphor, or no emollient, found a 41% reduction in nosocomial infections (Darmstadt, Saha et al. 2005) and a 26% reduction in neonatal mortality rates in the sunflower oil group. (Darmstadt, Saha et al. 2008) Many families from this same cohort perceived topical therapy with sunflower or Aquaphor to be better than mustard oil. (Ahmed, Saha et al. 2007)

Although studies have shown that topical application of vegetable oil may be beneficial to neonates, the choice of oil may be important. A study done using a mouse model showed accelerated skin barrier recovery in mice one hour after application of sunflower oil, with a sustained effect up to five hours while applications of mustard, olive, or soybean oils resulted in delayed recovery of skin barrier function. (Darmstadt, Mao-Qiang et al. 2002) Mustard seed oil showed the most detrimental effects, with sustained delay of barrier recovery for up to seven days. One possible reason for this delayed recovery is that mice treated with mustard seed oil showed structural changes in their epidermis, with flattened keratinocytes, apoptotic nuclei and nuclear envelope hypertrophy, and condensed heterochromatin. (Darmstadt, Mao-Qiang et al. 2002)

One way in which topical emollient application may be beneficial to neonates is by improved nutritional status. Nutritional deficiencies in infants can result from many sources such as inadequate diet, impaired absorption, high metabolic demands due to relatively large surface areas and rapid tissue growth, and defective metabolism of nutrients. Premature infants may be even more at risk because of a lack of nutrient stores. (Darmstadt 1998) Preterm neonates in Iran randomized to moderate pressure massage alone and the same massage using sunflower oil, showed improved weight gain in the group massaged with sunflower oil. (Fallah, Akhavan Karbasi et al. 2013) Another hospital-based study in Iran found a significant difference in weight gain between neonates massaged with coconut oil and both the group massaged without oil and the no massage group, but no difference between the massage only and no massage group. (Saeedi, Gholam et al. 2011) In addition, studies of very low birthweight preterm neonates (<1500g, <37 weeks gestational age and <1800g, <35 weeks gestational age) in India found infants receiving oil massage to have greater weight gain than those who received massage without oil or no massage. (Arora, Kumar et al. 2005; Kumar, Upadhyay et al. 2013)

Emollient therapy research to improve neonatal survival is a global priority. (Lawn, Zupan et al. 2006) Millions of infants in South Asia are exposed to the effects of mustard oil massage each year and it is unknown whether substituting sunflower seed oil changes any biological mechanisms that may improve neonatal health. We aimed to determine if and how certain biological mechanisms relating to nutritional status might differ between infants massaged with mustard seed oil and sunflower seed oil in rural Nepal. An examination of these possible mechanisms will facilitate characterization of the modes through which sunflower oil massage may improve neonatal health.

Methods

Settings and Population

This was a cluster-randomized community-based trial conducted by the Nepal Nutrition Intervention Project, Sarlahi (NNIPS). In 2011, the NNIPS surveillance area consisted of 26 Village Development Committees (VDCs) each encompassing nine government defined geopolitical units (wards), which were further divided into sectors based on population. This trial was nested within a larger parent trial on the Impact of Sunflower Seed Oil Massage on Neonatal Morbidity and Mortality in Nepal (NOMS). The original study population for the NOMS trial was all live born infants in households in 13 VDCs in Sarlahi District in rural Nepal. The Nepal Health Research Council and the Committee on Human Research of the Johns Hopkins Bloomberg School of Public Health approved this study.

Procedures and Design of the Parent Trial

Previous sector level mortality estimates were known for 9 of the 13 VDCs from prior research in this community and were used to perform restricted randomization in order to ensure balance of prior neonatal mortality risk. In the other 4 VDCs, sectors (clusters) were randomized with a computerized quasi-random number generator, stratified on VDC using blocks of 4, ensuring a geographical balance of the types of oil across the study area. Newborn infants were randomized within clusters (sectors) to receive either promotion of full body massage with sunflower seed oil or promotion of full body massage with mustard seed oil. A cluster-randomized design was chosen in order to minimize the chance of crossover or contamination of the intervention by providing each field worker with only one type of oil to promote in her area. It was not possible to blind field workers or mothers to the treatment, as mustard oil and sunflower oil have distinct colors and smells.

For both intervention and control groups, oil was purchased from Shiv Shakti in Jitpur, Nepal, approximately every 4-6 weeks and stored in sealed half-liter plastic packets at site headquarters at room temperature. The company was required to submit a sample for purity/quality to the Government of Nepal Food Inspection Laboratory in Hetauda, Makwanpur District, Nepal. A copy of this analysis was received from the Hetauda food lab for each distinct batch. Determination of the fatty-acid composition of the oils was done at Geo-Chem Laboratories PVT LTD (Mulund, Mumbai, India). This fatty-acid profile analysis was repeated twice per year. These analyses consistently estimated the linoleic acid levels between 58%-67% and 18%-22% in the sunflower and mustard oils, respectively.

Community-specific lists of married women of reproductive age were created. In order to rapidly identify new pregnancies, locally-resident female workers monitored the status of these women every 5 weeks. A woman identified as pregnant was approached for recruitment and consent for her infant's participation in the parent trial. Each woman participating in the study received a set of basic antenatal care interventions (e.g. tetanus toxoid, a clean delivery kit, iron-folate supplements, chlorhexidine cleaning solution for disinfecting the umbilical cord, iron folate, and deworming) and basic educational messages on antenatal and essential newborn care. The worker visited the women in late pregnancy (~28-32 weeks) to promote the use of either the sunflower seed oil or mustard seed oil, and provide the mother or other caretaker with a 100ml bottle of oil at that time. The mother or other caretaker initiated full body massage following the field worker's guidelines using the provided oil daily throughout the newborn period. The local project worker visited the homes daily during the first week of life to promote the continued use of the oil.

Immediately upon notification of the pregnancy, the local resident worker notified the supervisory staff and a birth assessment was conducted using standardized data collection forms recording information relating to late pregnancy morbidity, labor and delivery characteristics, birth assistants and practices, length of labor, maternal temperature, date and time of birth, sex, length and weight of infants, and immediate newborn care practices (thermal care, cord practices, early bathing and massage, breastfeeding initiation, etc.). In addition to the individual data obtained, household level data were collected at the time of enrollment of the mothers. This included information on socioeconomic factors, such as ethnicity, caste, household assets, ownership of materials or livestock, water sources, and family members who may be working overseas. Data were also collected on parental literacy, education, and birth history. After the initial birth visit, the birth team member completed newborn follow-up visits (NFF) on days 3, 7, 10, 14, 21, and 28 (for a total of 7 visits), where signs of infant morbidity and newborn care practices since prior visit were recorded. A 500ml bottle of oil was provided at the initial visit and on follow-up visits on days 10 and 21. The primary outcomes of NOMS are mortality within 28 days of birth, and possible severe infection in newborns.

Design of the Biological Mechanisms Sub-Study

This biological mechanisms sub-study included an extended set of measurements collected from a subset of infants participating in the main trial. The focus of data collection for this sub-study was on direct measurement of biological markers of infants built into the scheduled visits for the parent trial. Weight and length were used to assess nutritional status. Weight was taken at the initial birth assessment visit and at follow-up visits on days 3, 7, 10, 14, 21, and 28. Length was measured at the initial birth visit and at the 28-day follow-up visit.

A subset of 7 VDCs of the 13 original VDCs in the NOMS trial was selected to participate in the mechanisms study, which began in July 2012. An 8th VDC was added after 5 months and a 9th VDC was added in January 2013. The cluster-randomization of the parent trial was conserved for the mechanisms study. Among infants participating in the main study, determination of eligibility to additionally participate in the sub-study was done during the initial birth assessment visit, and was based on estimates of gestational age at birth. For this study, preterm infants (<37 weeks gestational age) were oversampled, aiming for approximately 50% of the sample to be preterm. During the study period (July 2012 until September 2013), the gestational age was estimated directly by the field worker using the date of last menstrual period estimated by the woman at the time of initial enrollment. Each birth team member had a list of the date of the mother's last menstrual period (LMP), which was used along with the date of birth to determine the gestational age of the infant. If the infant was born before week 37, one of the members of a specially trained team of field workers focused on the implementation of the mechanisms sub-study was contacted directly by mobile phone. Infants born on or after week 37 were listed by VDC in consecutive order (i.e. by birth date) on a pre-printed computer-generated blank form of 20 rows, 4 of which had been randomly selected for shading. Infants listed on a randomly shaded row were eligible for inclusion in the mechanisms study, thus selecting 4 out of every 20 (20%) of the full term infants for participation. All preterm (<37 weeks) infants and every 5th full term infant born in the mechanisms study area were eligible for enrollment in the sub-study. Infants were enrolled if consent was provided and the infant was met alive within 48 hours after birth. Newborns enrolled in the mechanisms study were visited in their homes 7 times by the birth assessment team, on days 1, 3, 7, 10, 14, 21 and 28 in order to collect weight and length (days 1 and 28 only) data. At these visits, the workers also asked questions relating to newborn care practices since the prior visit, including breastfeeding and other feeding practices and massage practices.

Measurement Methods

Newborns' weight measurements were taken without clothes using a Tanita portable digital infant scale (Tanita BD-585, Tokyo, Japan), with accuracy to ±10g. Length measurements were taken in centimeters using locally manufactured wooden length boards.

Statistical Analysis

The nested biological mechanisms study included a subset of the sample size of 29,620 infants required for the parent trial. Given that we hypothesized that the outcome measures (and the moderating effect(s) of the different oils, if any) may be more important in preterm infants than full term infants due to the immaturity of their skin, preterm infants were oversampled. A total sample size of 1000 infants was selected, equally stratified by 500 preterm and 500 full term infants. All power calculations assumed a design effect of 1.5, a 5% loss to follow-up, and a 5% Type 1 error. A design effect of 1.5 was assumed without prior knowledge of the true extent of correlation of the different measurements within clusters. The chosen sample size of 1000 infants enabled detection of a difference in mean values of weight and length of 0.5g or 0.5cm respectively, with between 71% and 100% power for standard deviations of 1.0 to 2.5. In addition, this sample size enabled us to detect a difference of 0.1 in the proportion of infants massaged with mustard seed oil versus sunflower oil with 2 SD or more below the normal mean weight-for-age or height-for-age with a power of 88-99% if the proportion of infants below -2 SD was between 0.5 and 0.1. The analyses presented here represent a preliminary analysis of the first 63.7% of the anticipated enrolled infants.

All analyses for these measurements were conducted using STATA v12 (College Station, TX). The comparability of the treatment groups in relation to their background characteristics was assessed to determine if there were any confounding variables. Possible confounders included: household demographics and socioeconomic status (SES), ethnic group, caste, maternal and paternal education levels, maternal age, reproductive history, labor and delivery characteristics (place of birth, length and type of labor, type and practice of birth assistants), newborn characteristics and newborn care practices (sex, birthweight, gestational age, small-for-gestational age (SGA) status, breastfeeding), and intervention exposure and compliance.

To examine whether there was an effect on weight, length, and z-scores for weight-for-age and height-for-age at different follow-up visit times due to the different oil groups, mixed-effects regression models with random intercepts were performed. Z-scores were calculated using WHO Anthro (version 3.2.2, January 2011). (WHO 2011) In all models, estimates of standard errors accounted for the clustered design. These models were also run to investigate differences between intervention groups in the change in weight between visits with 1, 2, and 3 visit increments (e.g. difference between weight at the birth visit and follow-up visit on day 3, the birth visit and the follow-up visit on day 7, etc.) and differences in the mean change in length between the birth visit and the follow-up visit on day 28 between the two oil groups. In addition, multi-level mixed-effects regression models with random intercepts, accounting for the clustered design and the repeated measures in the standard error estimates, were performed for the entire neonatal period and stratified by early (<7 days) and late (≥7 days) neonatal periods.

Multi-level models with an interaction between infant's age and oil group were also used to explore whether the rate of change in weight or length was different in the sunflower seed oil group. Mixed-effects logistic regression models with random-intercepts accounting for the clustered design in the standard error estimates were run to determine if there were differences in the proportion of infants who were stunted or underweight (<-2 z-scores below normal) at 28 days of age. Linear splines for infant's age at measurement were used where needed to account for nonlinearity, assessed through locally weighted regression smoothing. Sub-group analyses were conducted for preterm infants and small-for-gestational age (SGA) status for each measurement. Analyses followed an intention-to-treat approach for participating infants, regardless of the actual treatment provided.

Results

Between July 23, 2012 and September 30, 2013, there were a total of 1,876 full term and 409 preterm live born infants in the study area that were eligible for enrollment in the biological mechanisms study. Of these infants, 785 (376 full term and 409 preterm) were selected for enrollment (Figure 6-1). Of those selected, 148 (18.9%) died or were not met by the mechanisms study field worker before 48 hours after birth and 5 (0.6%) mothers refused their infant's participation in the study. A total of 632 newborns were enrolled in the mustard seed oil (N=324) and sunflower seed oil (N=308) clusters. In total, 271 (83.6%) and 262 (85.1%) of enrolled newborns completed 28 days of follow-up in the mustard seed oil and the sunflower seed oil groups respectively. In the sunflower oil group, 7 (2%) infants died and 39 (13%) permanently moved, most likely returning to the mother's maternal home or *maiti*, or were not met at their 28-day visit. In the mustard oil group, 6 (2%) infants died and 47 (14%) permanently moved or were not met at their 28-day visit.

Socioeconomic, household, and individual characteristics were well balanced between groups (Table 6-1), although infants in the sunflower oil group were more likely to be less than 2500 grams at first measurement at the initial birth assessment. In the sunflower seed oil group, 40.3% of infants were low birthweight compared to 33.9% in the mustard seed oil group. Overall prevalence of low birthweight was 36.7%, although this was not representative of the general population as preterm infants were oversampled. In the sunflower seed oil group, a greater proportion of infants were born to families who were of *Pahadi* (originating from the hills) ethnicity than in the mustard oil group and households in the sunflower seed oil group were less likely to have electricity. Both oil groups had similar proportions of infants that were small-for-gestational age (SGA). The overall prevalence of infants born SGA was 42.7%. The mustard seed oil group had a slightly higher proportion of infants who were born prematurely with 45.4% of newborns in the mustard seed oil group and 39.9% of newborns in the sunflower seed oil group born premature. Overall prevalence of prematurity in the study population was 42.7%. As these analyses were not completed on the total sample size, recruitment was still ongoing. Premature infants were being enrolled at a slower rate than full term infants, as our estimated proportion of preterm infants in this population (20%) was based on data from older studies. The actual proportion of infants who were born preterm in the study area during this study period was closer to 16%, accounting for the prevalence of preterm infants in the population used for these analyses being less than 50%.

The number of follow-up visits was similar for both treatment groups (Table 6-2). Both groups had very high percentages of newborns that had been massaged since the previous visit (or since birth, if visit 1). Reductions from full compliance (i.e. <100%) were more common in the sunflower seed oil group, especially on the first day of life (88.1% and 78.8% compliance in the mustard and sunflower oil groups, respectively), although greater than 98% of infants were massaged with oil in both groups prior to the first visit. Massage was frequent during the first week of life, and slightly more frequent in the mustard oil group: respondents reported means of 32.3 (±10.8) and 29.8 (±9.4) massages during the first week of life in the mustard and sunflower oil groups, respectively. In addition, the time from birth until the first weight measurement was nearly identical between oil groups (13.0 hours (±8.3) and 12.9 hours (±8.5) in the mustard and sunflower oil groups, respectively). The time between birth and first weight measurement were also similar between groups when stratified by term status.

Effect of Oil Group on Weight

Descriptive characteristics of weight by visit for each oil group stratified by term status are shown in Table 6-3. Infants in the sunflower oil group tended to have lower weight at each visit compared with those in the mustard oil group. This was most pronounced in the preterm infants where the mean birthweight (measured at the initial birth assessment visit) was 2562.6g (±473.1) and 2414.3g (±545.9) in the mustard oil and sunflower oil groups respectively. By visit 3 the mean weight of both groups had decreased, with a mean weight in the mustard oil group of 2530.1g (±506.8) and a mean weight in the sunflower oil group of 2391.6g (±534.8). Figures 6-2 through 6-7 show scatter plots of infants' weight by age with a locally weighted least squared regression (LOWESS) curve for each oil group and graphs with only the LOWESS curves, for the complete data set and stratified by term status. The curves of weight by age are very similar by oil group for the full term infants, while for preterm infants in the sunflower oil group, weight starts lower at the initial birth assessment visit and continues to remain lower throughout the neonatal period. The shapes of the curves are similar for all groups with a decrease in weight from day 0 to day 3 (-27.6g (±199.8) in the mustard oil group and -29.3g (±164.6) in the sunflower oil group) (results not shown) and increasing weight after day 3. Preterm infants in the mustard oil group had a difference in weight of -36.2g (±165.2) between their initial birthweight and weight at visit 3 and preterm infants in the sunflower group had a difference of -29.7g (±157.7) between these visits.

Table 6-4 displays regression results of the differences in oil groups on weight for different visits. The sunflower oil group tended to have lower weights for each visit when compared with the mustard oil group, however these were not statistically significant for any visits for either the complete data or for the full term group. In the preterm group, the sunflower oil group had significantly lower weights at the initial birth assessment visit and at follow-up visits on days 3 and 28 when compared to the mustard oil group. The sunflower oil group was estimated to have weights 148.3g (95% CI: 26.0-270.5), 138.5g (95% CI: 10.0-277.0), and 177.6g (95% CI: 7.1-348.1) lower compared to the mustard oil group at the initial birth visit and at follow-up visits on days 3 and 28 respectively. However, after controlling for the birthweight measurement, differences at visits 3 and 28 were no longer significant. Results from regression models investigating differences in weight change between visits by oil group are shown in Table 6-5. These results indicate no statistically significant differences in weight change between oil groups for any of the visits for the complete data and when stratified by term status, both when controlling for and not controlling for birthweight. There were also no statistically significant effects of oil group on weight change at 2 and 3 visit intervals. Oil group had no effect on weight by visit or change in weight between visits when stratified by SGA status.

Regression models showed no statistically significant differences in weight comparing the mustard oil and sunflower oil groups for the complete data or for the full term group for the complete neonatal period or when stratified by early and late neonatal periods. For the preterm group, the sunflower group's weight was estimated to be 147.4g (95% CI: 8.2-286.5) and 144.0g (95% CI: 17.8-270.2) lower than the mustard oil group's over the complete period and early neonatal period respectively, however after controlling for birthweight this effect was no longer statistically significant. Oil group had no effect on infants' weight in the late neonatal period for the preterm group. There was no effect of oil group on weight for any of the neonatal periods when stratified by SGA status.

The estimated average rate of change in weight per day over the neonatal period in the sunflower group compared to the mustard oil group was not statistically significantly different in the complete or full term group. However, after adjusting for birthweight, the rate at which weight increased in the preterm infants in the sunflower oil group was slower than that in the mustard oil group from day 4 to day 28. Between days 0 and 3, the rate at which preterm infants in the sunflower oil group lost weight was not statistically significant (15.1 g/day (95% CI: -32.6-2.3)). Between days 4 and 28, infants in the sunflower oil group were estimated to gain 28.4 g/day (95% CI: 26.8-30.1). Preterm infants in the mustard oil group were estimated to lose 18.7 g/day (95% CI: 3.9-33.5) between days 0 and 3 and gain 30.9 g/day (95% CI: 29.5-32.4) between days 4 and 28. While preterm infants in the sunflower oil group showed a trend of losing weight at a slower rate than infants in the mustard oil group between days 0 and 3, this was not statistically significant. However, between days 4 and 28 weight of preterm infants in the sunflower oil group was estimated to increase 2.6g (95% CI: 0.4-4.7) per day more slowly than preterm infants in the mustard oil group.

Estimated average rate of change in weight per day over different time periods in the neonatal period showed a significant difference in the estimated weight gain per day between days 14 and 28 for the complete group of 2.77g/day (95% CI: 0.12-5.4) less in the sunflower oil group when compared with the mustard oil group. In the preterm group between days 14 and 28, infants' weight in the sunflower oil group showed an estimated weight gain of 5.22g (95% CI: 1.11-9.33) less per day than the mustard oil group. After adjusting for birthweight the sunflower oil group had an estimated average weight gain of 2.85g (95% CI: 0.21-5.49) and 5.22g (95% CI: 1.11-9.33) less per day in the complete group and the preterm group respectively when compared with the mustard oil group. There were no statistically significant differences in average rate of change in weight per day when stratified by SGA status.

Effect of Oil Group on Length

Descriptive characteristics of infants' length at the initial birth visit and day 28 follow-up visit are shown in Table 6-6. Mean lengths at both the initial visit and day 28 visit were very similar between oil groups. In the preterm group, infants in the sunflower oil group had estimated mean lengths of 46.3cm (±3.0) and 50.8cm (±3.0) at the initial birth visit and at follow-up visit on day 28, respectively, while preterm infants in the mustard oil group had mean lengths of 46.8cm (±2.9) and 51.2cm (±3.0) at the initial birth visit and at visit 28, respectively. Figures 6-8 to 6-10 show distributions of infants' length by visit for each oil group for the complete data and stratified by term status, showing similar distributions for both oil groups.

Regression models showed no statistically significant effect of oil group on infants' length at the initial or day 28 visit for the complete group or when stratified by term status (Table 6-7), although infants in the sunflower group tended to be smaller at both visits. There was also no statistically significant effect of oil group on the difference in length gain between the two visits, or on the average change in length per day. This was also true when stratified by SGA status and when controlling for birthweight.

Effect of Oil Group on Z-score

The proportion of infants in this population who were underweight or stunted at visit 28 is shown in Table 6-8. The sunflower oil group had greater proportions of infants who were underweight and stunted for the complete data set and for both full term and preterm groups, although the full term groups were very similar. The proportion of preterm infants in the sunflower oil group who were underweight or stunted was 47.7% and 32.7%, respectively compared with 35.5% and 27.4% who were underweight or stunted in the mustard oil group. However, logistic regression models indicated that oil group had no effect on the odds of being underweight or stunted at 28 days for any of the term stratification groups (results not shown). This was also true when the data was stratified by SGA status.

Descriptive characteristics for the average z-scores for weight-for-age and height-for-age at follow-up visit 28 are shown in Table 6-9. Both oil groups have lower mean z-scores for weight-for-age than for height-for-age. The sunflower oil group had lower mean z-scores for both weight-for-age and height-for-age for the complete data set and also for both full term and preterm groups. Regression analyses indicated preterm infants in the sunflower oil group had mean weight-for-age z-scores 0.37 (95% CI: 0.0084-0.74) lower when compared with the mustard oil group at visit 28 (results not shown). This was however no longer statistically significant after adjusting for birthweight. Other differences in z-scores were not statistically significant nor were differences when stratified by SGA groups.

Discussion

These data do not provide evidence that full body oil massage with sunflower oil improves neonates' nutritional status when compared to full body oil massage with mustard oil in this population of neonates in rural Nepal. This is in contrast to other studies, which have found improved weight and/or length gain in infants massaged with oils high in linoleic acid. (Arora, Kumar et al. 2005; Vaivre-Douret, Oriot et al. 2009; Fallah, Akhavan Karbasi et al. 2013; Kumar, Upadhyay et al. 2013) However, these studies differed from our study in several important ways. These were all hospital-based studies with neonates who were generally more vulnerable and who were less than 35 weeks of age or of very low birthweight. In addition, these studies all used a standardized massage technique, using moderate pressure for a prescribed amount of time. In contrast, our study was done in a community setting, where most women in this population give birth in the home and the caretakers performed massage in a traditional (i.e. quite vigorous) way. These differences in study design may have led to differences in outcomes. For example, full body oil massage with an oil high in linoleic acid may only improve nutritional status in infants who are either very premature or of very low birthweight, both of whom are unlikely to survive in a rural low-resource community setting. Fewer than 40 infants in our study population were less than 32 weeks gestational age, which may have made a difference in oil groups unable to be detected.

In addition, very preterm infants have a more permeable skin barrier, which may allow more transcutaneous absorption of the oils. (Cartlidge 2000)

Type of massage may also affect a neonate's nutritional status. Studies of infant massage using moderate pressure have shown a decrease in stress behavior and cortisol levels and an increase in weight gain compared with infants who are not massaged. (Field, Diego et al. 2010) However, infant massage in this population is quite vigorous, leaving infants stressed and agitated. This could lead to an increase in cortisol levels, which may influence weight gain. (Field, Diego et al. 2010)

This study found that preterm infants massaged with sunflower oil had slower weight gain after day 3 than infants massaged with mustard seed oil. We are uncertain as to why this may have occurred, however it could be due to unmeasured confounders in this population. Although it is unclear why there may be slower weight gain in preterm infants in the sunflower oil group, this small difference in weight gain (about 3 grams per day less than the mustard oil group) is unlikely to be clinically significant.

This study only compared the differences in nutritional status between infants massaged with an improved oil (sunflower oil) and the traditional oil (mustard oil). One of the limitations of this study was that there were no control groups for infants massaged without oil or infants who did not receive massage. Therefore, the beneficial nutritional effects of oil massage found in other studies, could be due to massage alone, or massage with any type of oil and not due to the type of oil used. A study by Ang et al. showed infants 28-33 weeks who were massaged had an increase in mean final weight and daily weight gain compared with infants who were not massaged. (Ang, Lua et al. 2012) In addition, a meta-analysis showed massage improved daily weight gain by

5.32g. (Wang, He et al. 2013) Another study of one-month old infants reported less stress behaviors, lower salivary cortisol levels, and increased vagal activity in infants who were massaged with oil compared with those who were massaged without oil. (Field, Schanberg et al. 1996) Increased vagal activity, which increases gastric motility, may lead to an increase in food absorption hormones such as insulin and may be one of the underlying mechanisms for greater weight gain in massaged infants. (Field, Diego et al. 2011) An increase in IGF-1 through an independent pathway that is not related to vagal activity was also related to weight gain perhaps because of fomenting muscle and bone development. (Field, Diego et al. 2011)

Although the cluster-randomized design and the large sample size with an oversampling of preterm infants was a strength of this study, unfortunately balance was not achieved between the birthweights of premature infants in the mustard oil and sunflower oil groups. On average, the premature infants in the sunflower oil group were of lower birthweight than the premature infants in the mustard oil group. This should not have influenced our results however, as we were able to adjust for birthweight during analyses.

In addition, using recalled date of last menstrual period (LMP) in order to determine gestational age may have resulted in misclassification of infants as either preterm or full term. This method has been shown to be less accurate than methods using ultrasound. A study done in a tertiary-care hospital in Bangladesh found that LMP underestimated gestational age by one day compared with estimation from ultrasound. (Rosenberg, Ahmed et al. 2009) In addition, a study done at a hospital in Pakistan found that only 65% of estimated gestational age of reported LMP were within 7 days and only 82% were within 14 days when compared to gestational age estimates using ultrasound.

(Jehan, Zaidi et al. 2010) Despite the problems of the uncertainty of LMP date, in this community setting in rural Nepal, the low-cost and simple method of reported LMP is the best option for estimating gestational age. Also, LMPs from our study were collected as soon as a pregnancy was identified (following-up women every 5 weeks), so recall bias would be minimized. In order to determine the accuracy of using recalled LMP to estimate gestational age in this population and to determine the direction of possible misclassification, research should be done on a small number of women comparing LMP estimates to estimation from ultrasound. Analyses should also be performed limiting our preterm group to those <34 weeks in order to reduce some of the possible misclassification problems and to determine if there are effects of the oils on nutritional status in infants who are more premature.

Further research should be conducted in this population investigating whether massage with emollients has any effect on nutritional status when compared to massage without emollients and to no massage. It should also be investigated whether changing the way in which an infant is massaged (e.g. vigorous versus gentle massage) has any impact on nutritional status. Both changing the way in which an infant is massaged and whether an infant is massaged would be very difficult in this community setting in rural Nepal, as neonatal oil massage is a nearly universal cultural practice. (Mullany, Darmstadt et al. 2005) However, a smaller group of neonates in an urban area may be more willing to accept these behavioral change interventions. Another possibility in this rural community is to enroll older infants past the age when it is considered a cultural necessity to massage the infants, in order to get control groups of massage without oil or no massage.

It is important to have a better of understanding of the underlying mechanisms of how and why emollient therapy can improve neonatal health outcomes in low-resource settings, because the optimization of health benefits of oils depends upon the understanding of which biological mechanisms are impacted by the use of different oils. Further research on possible differences in immune function, skin integrity, and bacterial colonization in infants massaged with sunflower oil compared to those massaged with mustard seed oil should also be considered.

Chapter 6 Tables and Figures

Table 6-1: Baseline Maternal, Socioeconomic, Household, and Newborn Care Characteristics by Intervention Group

	Oil Randomization Group			
	Mustard Oil		Sunflower Oil	
	N	(%)	N	(%)
Number of Clusters	56		58	
Total number of infants	324		308	
Ethnic Group				
Pahadi	15	4.8	29	9.5
Madeshi	300	95.2	277	90.5
Maternal literacy				
Not literate	229	71.3	220	71.2
Literate	92	28.7	89	28.8
Paternal literacy				
Not literate	153	47.7	141	45.6
Literate	168	52.3	168	54.4
Maternal Education				
None	229	71.3	221	71.5
1-5 yrs	30	9.4	29	9.4
6-10 yrs	47	14.6	47	15.2
>10 yrs	15	4.7	12	3.9
Paternal Education				
None	147	45.8	142	46.0
1-5 yrs	48	15.0	48	15.5
6-10 yrs	100	31.2	105	34.0
>10 yrs	26	8.1	14	4.5
Household assets§				
0 or 1 asset(s)	22	7.0	22	7.2
2-5 assets	135	42.9	143	46.7
6-10 assets	150	47.6	133	43.5
>10 assets	8	2.5	8	2.6
Electricity				
No	112	35.6	140	45.8
Yes	203	64.4	166	54.3
Maternal Age				
<18 yrs	43	13.4	41	13.3
18-24 yrs	177	55.1	180	58.3
25-29 yrs	72	22.4	63	20.4
30-34 yrs	23	7.2	13	4.2
>=35 yrs	6	1.9	12	3.9

Table 6-1: Baseline Maternal, Socioeconomic, Household, and Newborn Care Characteristics by Intervention Group (continued)

	Oil Randomization Group			
	Mustard Oil		Sunflower Oil	
	N	(%)	N	(%)
Gravidity				
None	83	25.9	83	26.9
1-2	143	44.6	152	49.2
3-4	85	26.5	70	22.7
>5	10	3.1	4	1.3
Parity				
None	88	27.4	91	29.5
1-2	135	42.1	136	44.0
3-4	82	25.6	73	23.6
>5	16	5.0	9	2.9
Antenatal Care Visits				
No ANC	90	28.0	92	29.8
1-2 ANC visits	122	38.0	109	35.3
3-4 ANC visits	94	29.3	96	31.1
>=5 ANC visits	15	4.7	12	3.9
Location of delivery				
Home	163	50.8	160	51.8
Maiti	57	17.8	52	16.8
HP/Clinic	54	16.8	58	18.8
Hospital	36	11.2	32	10.4
On way to facility	11	3.4	7	2.3
Length of labor				
<5 hrs	147	45.8	149	48.4
5-14 hrs	144	44.7	118	38.3
15-19 hrs	11	3.4	16	5.2
20-24 hrs	10	3.1	14	4.6
>24 hrs	9	2.8	11	3.6
Complications during delivery				
No	267	83.2	246	80.1
Yes	54	16.8	61	19.9
Delivery Assistant				
No one/Family/Neighbors	81	25.3	78	25.2
TBA/Chamain	57	17.8	61	19.7
CHV/VHW/MCH Worker	14	4.4	8	2.6
ANM/HA/CMA/Staff Nurse	84	26.3	86	27.8
Local doctor	79	24.7	73	23.6
MBBS doctor	5	1.6	3	1.0

Table 6-1: Baseline Maternal, Socioeconomic, Household, and Newborn Care Characteristics by Intervention Group (continued)

	Oil Randomization Group			
	Mustard Oil		Sunflower Oil	
	N	(%)	N	(%)
Sex				
Male	169	51.8	160	51.4
Female	157	48.2	151	48.6
Gestational Age				
<32 wks	20	6.3	17	5.6
32-36 wks	124	39.1	104	34.3
37-41 wks	148	46.7	157	51.8
>=42 wks	25	7.9	25	8.3
Birthweight				
<1500g	4	1.3	8	2.6
1500-2499g	104	32.6	115	37.7
>2500g	211	66.1	182	59.7
SGA Status				
AGA	185	58.0	172	56.4
SGA 3-10%	62	19.4	57	18.7
SGA 3%	72	22.6	76	25.0
Breastfed since birth at first visit				
No	54	16.6	42	13.5
Yes	272	83.4	269	86.5
Hours before breastfeeding initiation				
<1 hr	87	32.3	74	27.8
1-2 hrs	154	57.2	151	56.8
3-4 hrs	15	5.6	20	7.5
>=5 hrs	13	4.8	21	7.9
Infant received colostrum				
No	30	11	27	10
Yes	241	88.6	242	90
Gestational Age (wks) (mean, SD)	37.5	(3.7)	37.9	(3.5)
Birthweight (g) (mean, SD)	2639.4	(465.6)	2582.8	(501.3)

§: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home

Table 6-2: Intervention Coverage by Intervention Group

	Mustard Oil		Sunflower Oil	
	N	%	N	%
# of follow-up visits* (mean, SD, range)	6.3 (1.5) 1-7		6.23 (1.6) 1-7	
Hours from birth until initial weight measure (mean, SD, range)	13.0 (8.3) 1.3-45.3		12.9 (8.5) 0.9-42.6	
# of times massaged in first week (mean, SD, range)	32.3 (10.8), 1-83		29.79 (9.36) 3-63	
Avg # of times massaged per day in first week (mean, SD, range)	4.7 (1.5) 1-11.9		4.31 (1.27) 1.5-9	
Massaged since last visit§				
Visit 1	320	98.8	307	99.7
Visit 3	313	100	296	99.7
Visit 7	307	99.0	287	98.6
Visit 10	295	98.0	281	96.9
Visit 14	299	98.7	284	97.6
Visit 21	291	99.3	276	97.5
Visit 28	273	99.6	260	99.2
Massaged with NNIPS oil during last massage				
Visit 1	282	88.1	242	78.8
Visit 3	311	99.3	283	95.6
Visit 7	299	97.4	261	90.9
Visit 10	290	98.3	263	93.6
Visit 14	287	96.0	261	91.9
Visit 21	282	96.6	256	92.8
Visit 28	263	96.7	232	89.2

*Max number of household visits is 7; § At visit 1 this is massaged since birth

Table 6-3: Descriptive Characteristics of Weight by Visit and Intervention Group

Visit Number	Mustard Oil				Sunflower Oil			
	N	n	Mean (g)	SD (g)	N	n	Mean (g)	SD (g)
Complete Data Set								
1	324	56	2633.6	455.4	308	57	2578.3	501.1
3	312	56	2606.4	484.8	295	57	2555.9	501.0
7	308	56	2762.8	493.3	290	57	2717.0	527.8
10	300	56	2851.3	506.4	289	57	2796.0	549.1
14	302	56	2972.3	530.4	289	57	2936.2	569.6
21	292	56	3196.7	569.4	283	57	3164.1	595.4
28	273	56	3446.1	594.5	262	57	3359.7	632.6
Full Term								
1	175	52	2712.8	416.5	184	52	2703.5	416.5
3	166	51	2696.4	435.3	177	52	2681.6	415.2
7	168	51	2850.0	434.3	176	52	2843.4	432.4
10	162	51	2968.8	432.5	173	51	2940.1	456.3
14	164	50	3089.2	450.7	176	51	3069.5	476.7
21	159	50	3324.0	490.0	171	52	3316.3	507.8
28	146	49	3582.5	488.8	153	51	3539.2	531.3
Preterm								
1	144	53	2562.6	473.1	121	47	2414.3	545.9
3	141	53	2530.1	506.8	115	47	2391.6	534.8
7	137	53	2672.6	535.5	111	47	2552.7	575.3
10	134	53	2734.9	543.0	114	47	2599.4	591.7
14	134	53	2854.9	576.2	111	47	2754.3	614.0
21	130	52	3062.5	613.9	110	46	2952.5	628.6
28	124	52	3305.9	661.2	107	45	3128.3	663.1

N=number of observations, n=number of clusters, SD=standard deviation

Table 6-4: Regression Results of Intervention Group on Weight by Visit Number

Visit Number	N	n	Coefficient (g)	SE (g)	95% CI (g)
Complete Data Set					
1	632	113	-58.0	39.6	-135.6-19.6
3	607	113	-50.5	39.9	-128.8-27.8
7	598	113	-45.7	41.7	-127.4-36.0
10	589	113	-67.0	48.4	-161.9-27.9
14	591	113	-43.0	47.7	-136.6-50.5
21	575	113	-41.8	53.3	-146.4-62.8
28	535	113	-90.3	55.7	-199.4-18.8
Full Term					
1	359	104	-9.3	43.9	-95.3-76.6
3	343	103	-14.9	45.8	-104.6-74.9
7	344	103	-6.6	46.6	-97.9-64.7
10	335	102	-28.7	48.5	-123.7-66.4
14	340	102	-19.7	50.5	-119.8-80.5
21	330	102	-19.9	58.6	-134.7-94.8
28	299	100	-44.9	59.9	-162.2-72.4
Preterm					
1	265	100	-148.3*	62.4	-270.5- -26.0
3	256	100	-138.5*	65.5	-277.0- -10.0
7	248	100	-119.9	70.4	-257.9-18.2
10	248	100	-135.5	71.8	-276.2-5.3
14	245	100	-100.5	75.9	-249.2-48.2
21	240	98	-110.0	80.1	-266.9-46.9
28	231	97	-177.6*	87.0	-348.1- -7.1
Preterm adjusted for birthweight					
3	256	100	9.4	20.6	-30.9-49.7
7	248	100	22.7	49.0	-20.0-65.3
10	248	100	22.3	26.5	-29.5-74.2
14	245	100	16.6	29.9	-42.0-75.3
21	240	98	-1.1	33.4	-66.6-64.4
28	231	97	-40.9	41.3	-121.9-40.1

N=number of observations, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE= standard errors, 95% CI=95% confidence interval, *=p<0.05

Table 6-5: Regression Results of Change in Weight Between Visits by Intervention Group and Visit Number

Visit Interval	N	n	Coefficient (g)	SE (g)	95% CI (g)
Complete Data Set					
1-3	607	113	-1.7	14.9	-30.9-27.5
3-7	589	113	12.2	12.9	-13.1-37.5
7-10	577	113	-5.7	9.9	-25.1-13.8
10-14	570	113	-1.6	10.6	-22.0-19.5
14-21	555	113	7.7	13.5	-18.8-34.2
21-28	522	113	-16.2	12.8	-41.2-8.9
Full Term					
1-3	343	103	-11.3	21.3	-53.0-30.4
3-7	338	103	21.2	17.6	-13.3-55.8
7-10	332	102	-6.8	13.3	-32.9-19.2
10-14	328	102	4.6	11.2	-17.3-26.5
14-21	320	101	18.7	15.2	-11.1-48.4
21-28	291	100	-7.6	17.1	-41.1-25.8
Preterm					
1-3	256	100	6.5	20.4	-33.4-46.5
3-7	245	100	-1.9	18.8	-38.7-34.9
7-10	240	100	-7.7	15.0	-37.1-21.7
10-14	236	100	-5.3	18.6	-41.8-31.2
14-21	230	98	-21.1	20.2	-59.8-19.6
21-28	226	97	-28.3	19.6	-66.7-10.2
Preterm adjusted for birthweight					
1-3	256	100	9.4	20.6	-30.9-49.7
3-7	245	100	6.5	18.9	-30.6-43.5
7-10	240	100	-9.1	15.1	-38.8-20.5
10-14	236	100	2.0	18.1	-33.5-37.4
14-21	230	98	-16.4	20.1	-55.9-23.1
21-28	226	97	-24.8	19.6	-63.2-13.6

N=number of observations, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE= standard errors, 95% CI=95% confidence interval

Table 6-6: Descriptive Characteristics of Length by Visit and Intervention Group

Visit Number	Mustard Oil				Sunflower Oil			
	N	n	Mean (cm)	SD (cm)	N	n	Mean (cm)	SD (cm)
Complete Data Set								
1	324	56	47.5	2.7	308	57	47.2	2.7
28	272	56	52.0	2.7	261	57	51.6	2.9
Full Term								
1	175	52	48.2	2.0	184	52	47.9	2.1
28	145	49	52.7	2.2	153	51	52.3	2.5
Preterm								
1	144	53	46.8	2.9	121	47	46.3	3.0
28	124	52	51.2	3.0	106	44	50.8	3.0

N=number of observations, n=number of clusters, SD=standard deviation

Table 6-7: Regression Results of Intervention Group on Length by Visit Number

Visit Number	N	n	Coefficient (cm)	SE (cm)	95% CI (cm)
Complete Data Set					
1	632	113	-0.3	0.3	-0.8-0.2
28	533	113	-0.4	0.3	-0.9-0.1
Full Term					
1	359	104	-0.3	0.2	-0.7-0.2
28	298	100	-0.5	0.3	-1.1-0.2
Preterm					
1	265	100	-0.6	0.4	-1.3-0.2
28	230	96	-0.4	0.4	-1.2-0.3

N=number of observations, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE= standard errors, 95% CI=95% confidence interval

Table 6-8: Stunting and Underweight on Visit 28 by Intervention Group

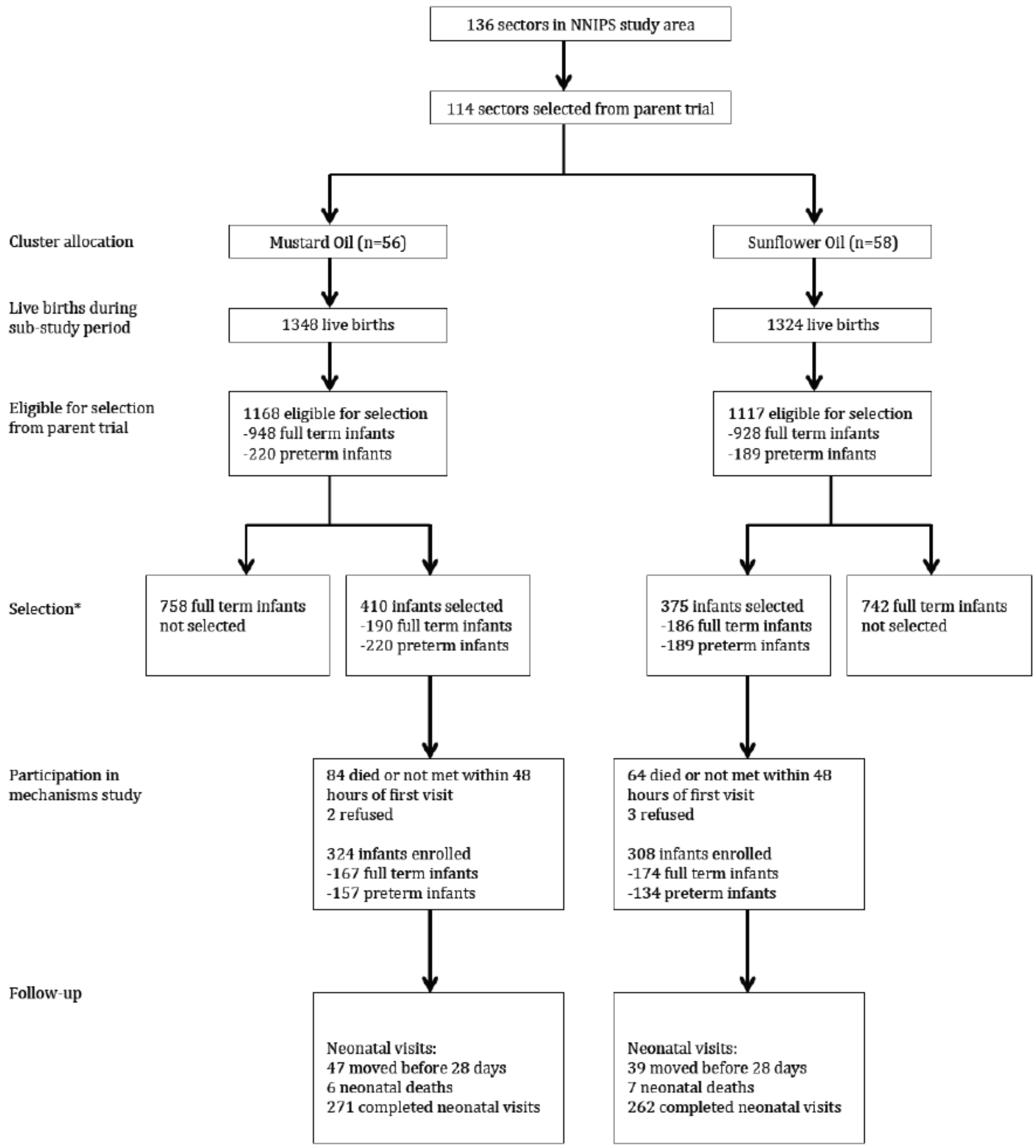
	Oil Randomization Group			
	Mustard Oil		Sunflower Oil	
	N	(%)	N	(%)
Complete Data Set				
Underweight	77	28.2	85	32.4
Stunted	54	19.8	59	22.5
Full Term				
Underweight	30	20.5	32	20.9
Stunted	18	12.3	23	15.0
Preterm				
Underweight	44	35.5	51	47.7
Stunted	34	27.4	35	32.7

Table 6-9: Descriptive Characteristics of Z-scores on Visit 28 by Intervention Group

	Mustard Oil				Sunflower Oil			
	N	n	Mean Z-score	SD	N	n	Mean Z-score	SD
Complete Data Set								
Weight-for-Age	273	56	-1.5	1.2	262	57	-1.7	1.3
Height-for-Age	273	56	-2.0	1.3	262	57	-1.2	2.1
Full Term								
Weight-for-Age	146	49	-1.2	0.9	153	51	-1.3	1.0
Height-for-Age	146	49	-0.6	1.1	153	51	-0.8	1.3
Preterm								
Weight-for-Age	124	52	-1.8	1.4	107	45	-2.2	1.4
Height-for-Age	124	52	-1.3	1.5	107	45	-1.8	2.7

N=number of observations, n=number of clusters, SD=standard deviation

Figure 6-1: Study Flow Diagram



*20% of full term neonates were randomly selected for inclusion and all preterm neonates were selected for inclusion in the sub-study.

Figure 6-2: Weight by Infant's Age and Intervention Group-Complete Data

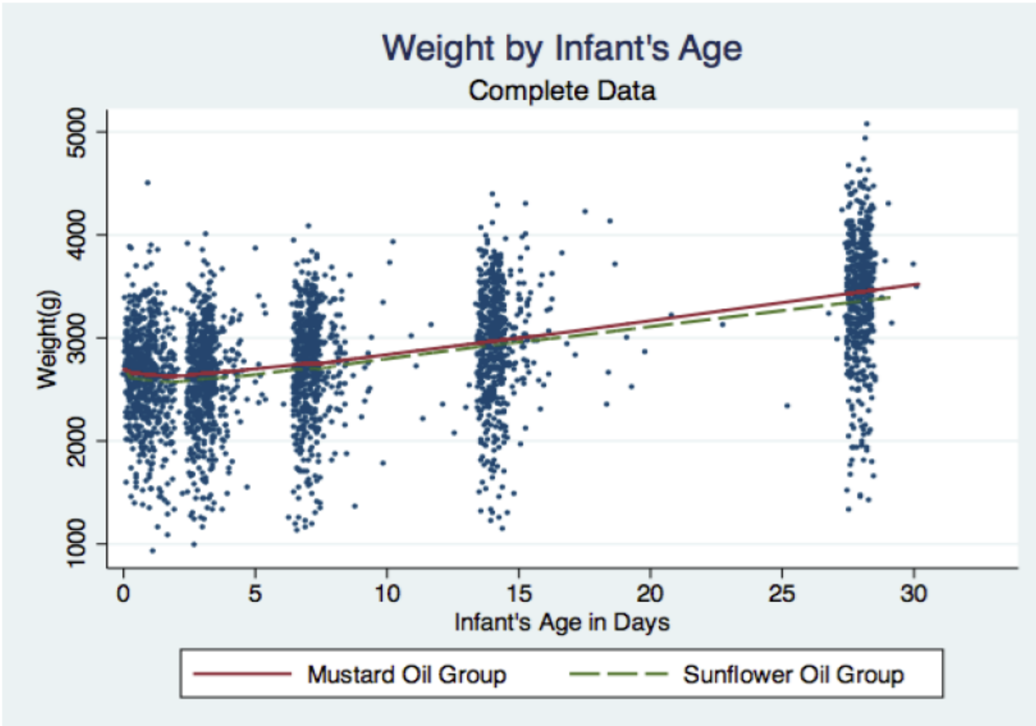


Figure 6-3: Weight by Infant's Age and Intervention Group-Complete Data LOWESS Curves

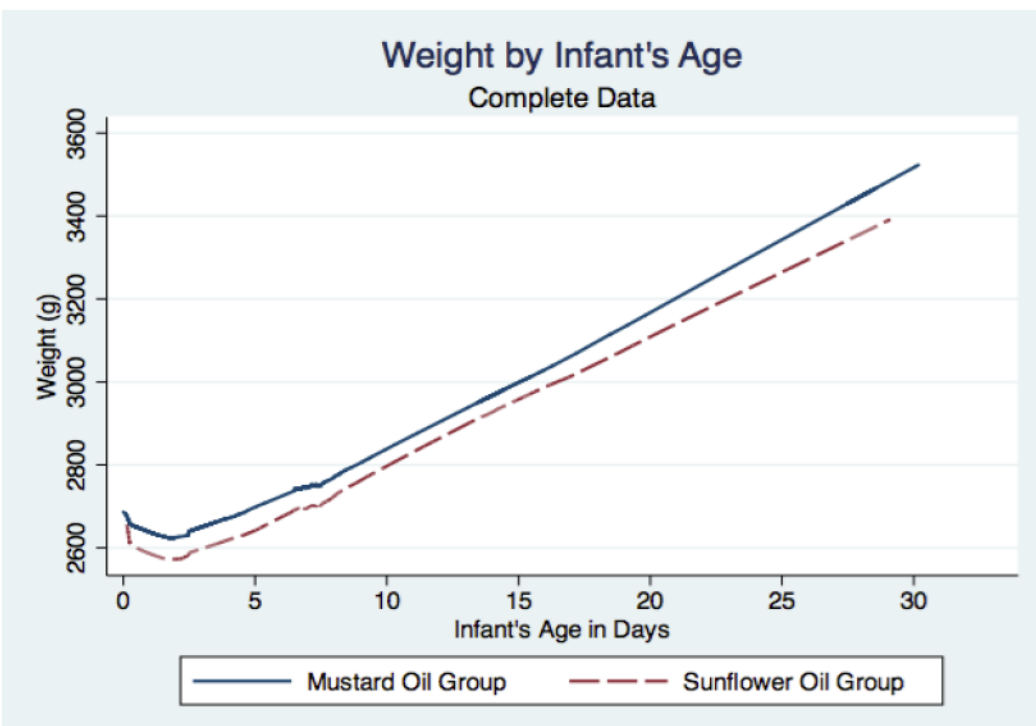


Figure 6-4: Weight by Infant's Age and Intervention Group-Full Term

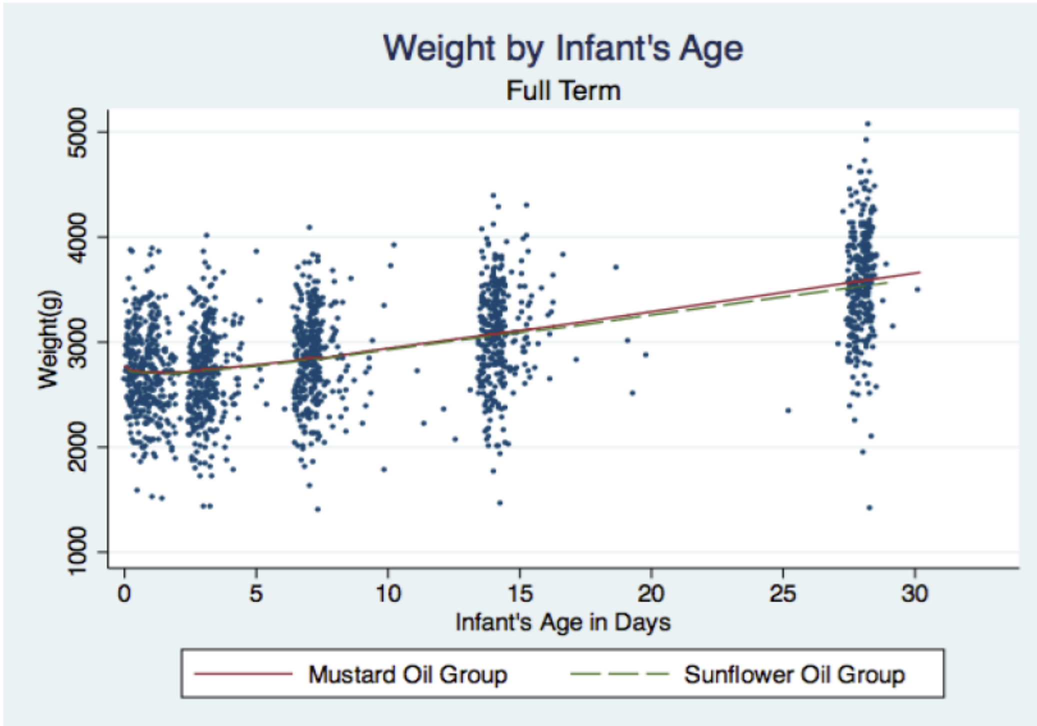


Figure 6-5: Weight by Infant's Age and Intervention Group-Full Term LOWESS Curves

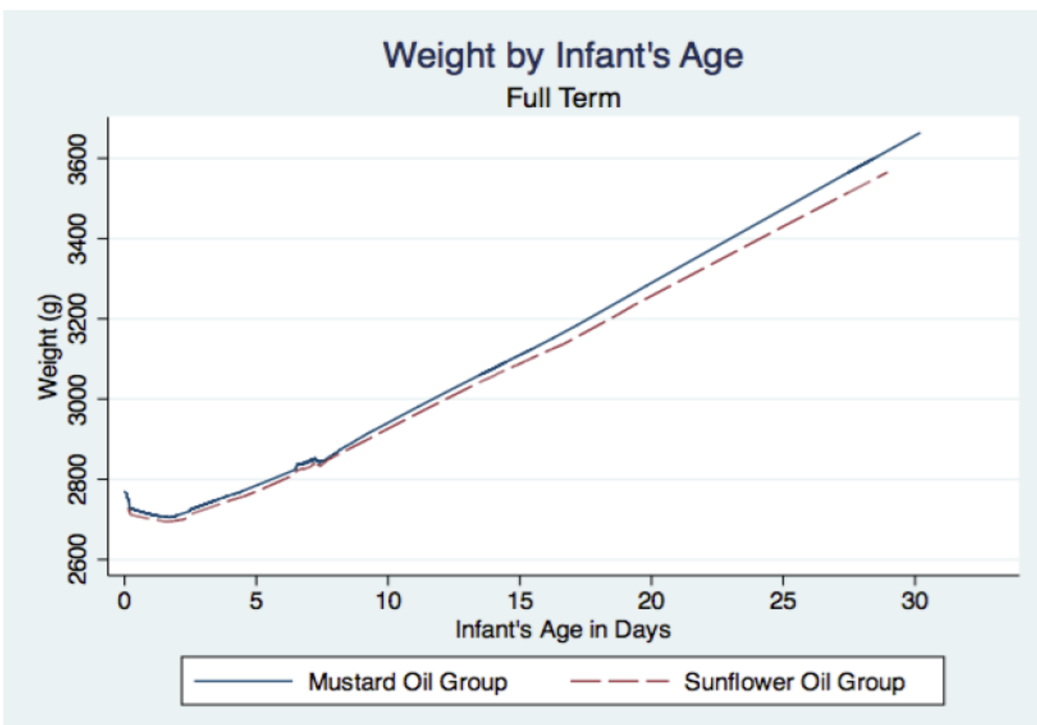


Figure 6-6: Weight by Infant's Age and Intervention Group-Preterm

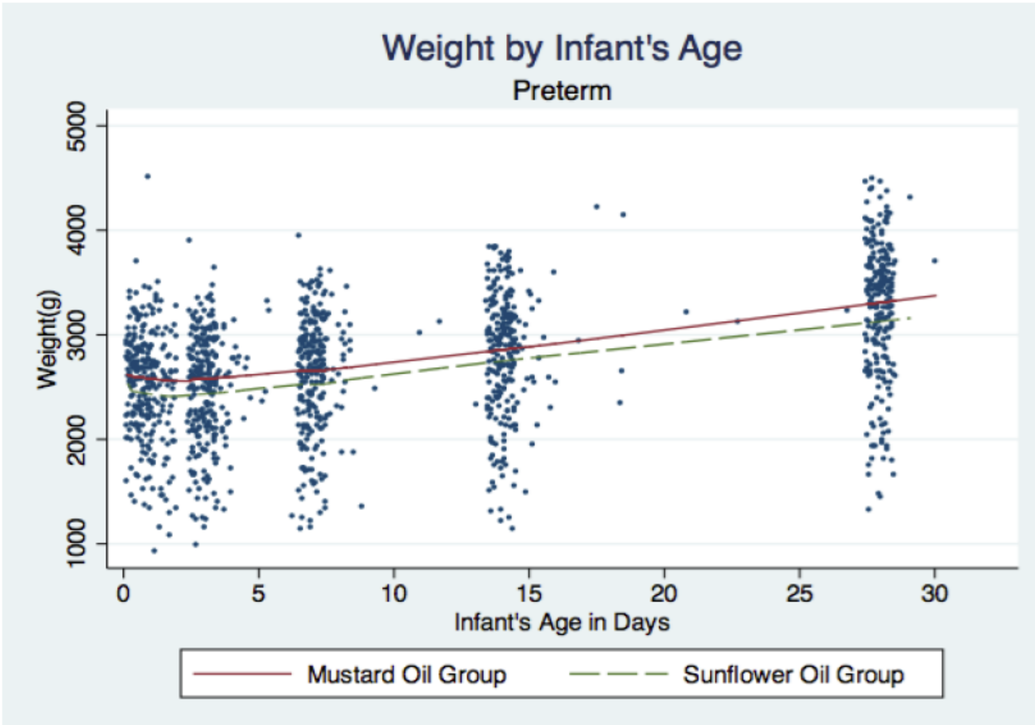


Figure 6-7: Weight by Infant's Age and Intervention Group-Preterm LOWESS Curves

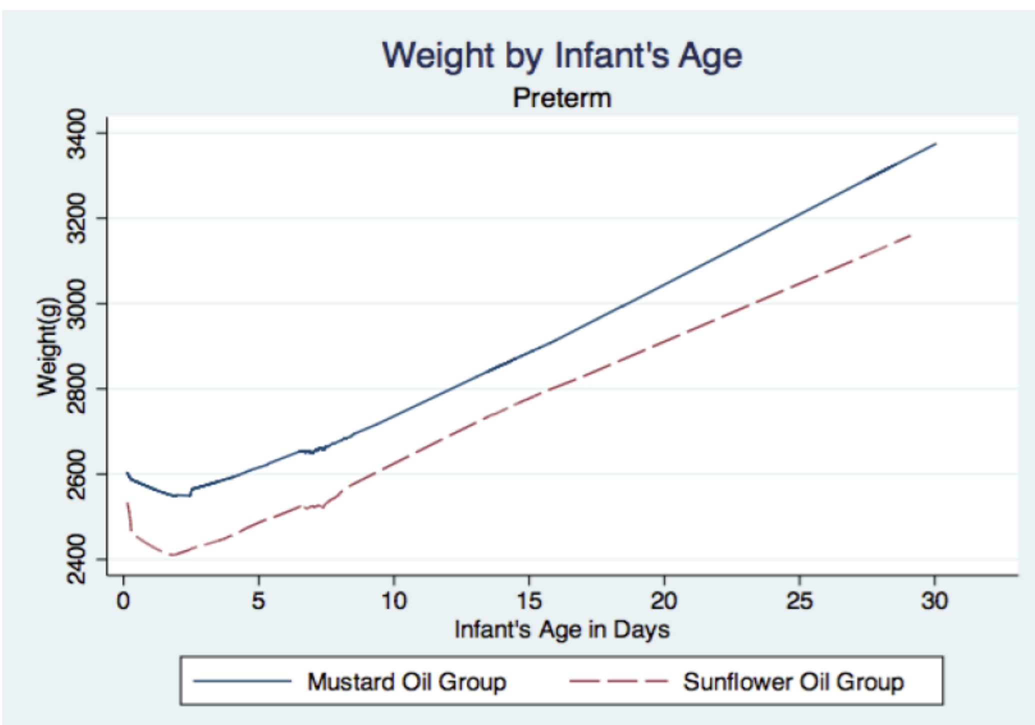


Figure 6-8: Length by Visit and Intervention Group-Complete Data

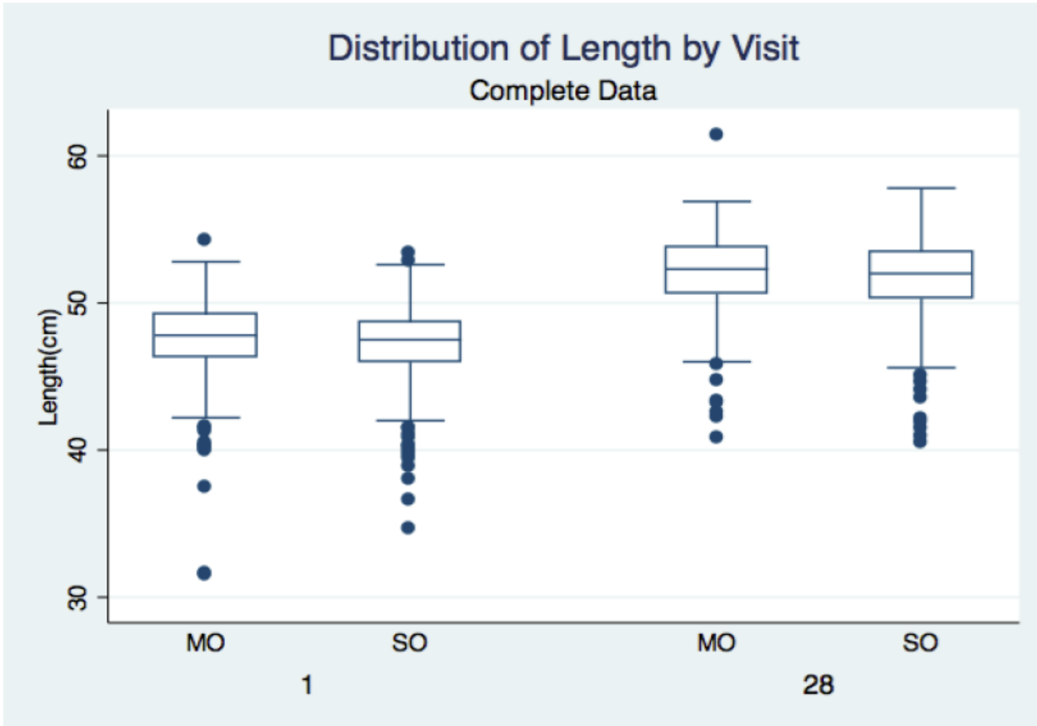


Figure 6-9: Length by Visit and Intervention Group-Full Term

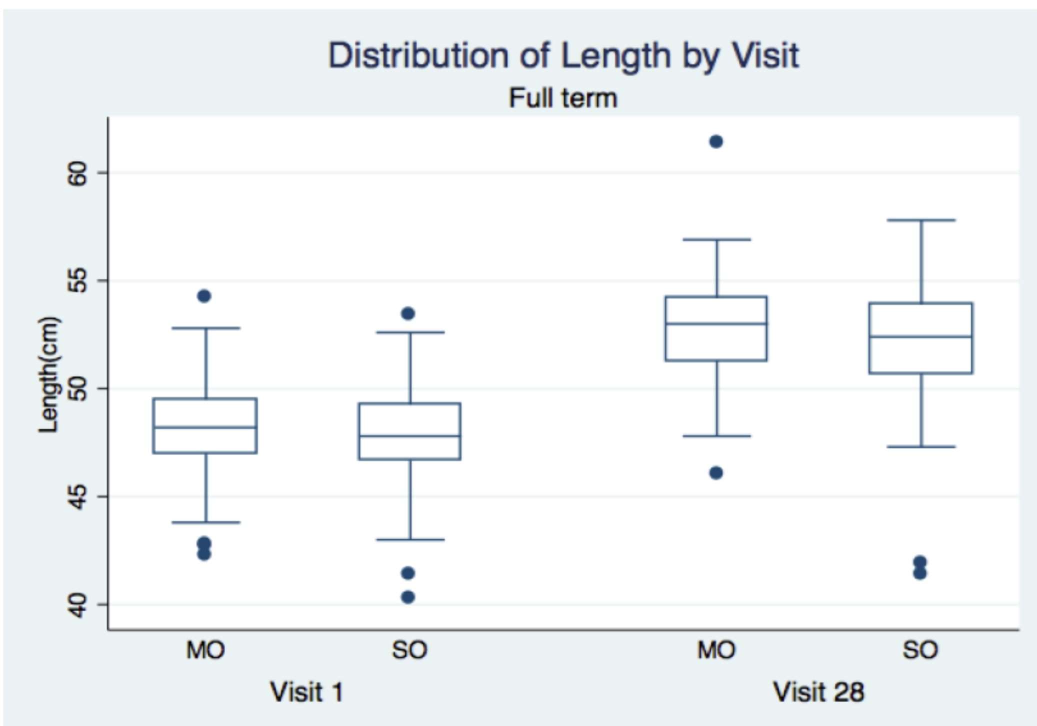
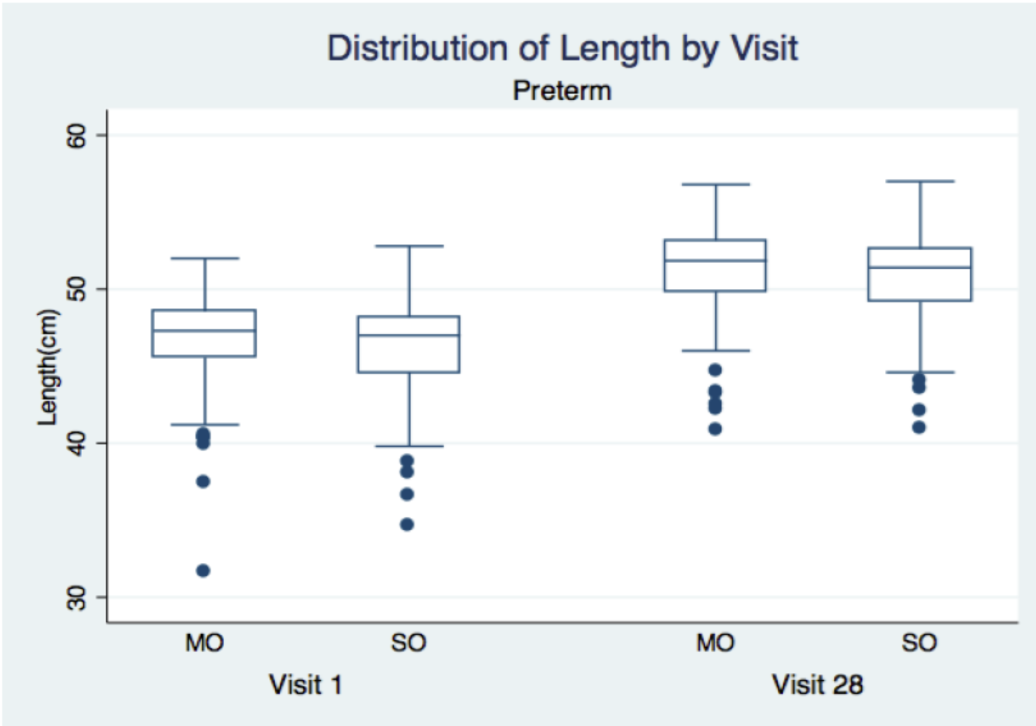


Figure 6-10: Length by Visit and Intervention Group-Preterm



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Chapter 7 Discussion and Recommendations for Further Research

Risk Factors Associated with Skin Integrity and Barrier Function

This research examined risk factors associated with skin integrity and barrier function and how these measurements change over the neonatal period in a community where mothers typically perform full body neonatal oil massage.

In this population, transepidermal water loss (TEWL) measures were much higher at birth than TEWL measures from other studies. (Visscher, Odio et al. 2009; Kelleher, O'Carroll et al. 2013) In addition, TEWL values in this population increased over the entire neonatal period, with the greatest rate of increase occurring over the first 3 days. This is in contrast to other studies that have shown full term infants' TEWL values to be similar to or lower than adult values and then remain stable over the first month of life, while values in preterm infants start higher, but decrease relatively quickly during the first few days. (Agren, Sjörs et al. 1998; Kalia, Nonato et al. 1998) However, these studies were done in climate-controlled hospital settings, which could explain some of the differences when comparing our results. Our study was conducted in women's homes in an environment of consistently very high relative humidity, with average daily humidity over 75%. Not surprisingly, increases in relative humidity were also associated with increases in TEWL, most likely due to sweat being unable to evaporate at high humidity. (Foster, Hey et al. 1969) However, increases in temperature were associated with decreases in TEWL. The reason for this could be that increases in temperature causes increased blood flow to the skin, which is needed for maturation and repair of the skin barrier. Therefore higher temperatures could lead to decreased TEWL, indicating improved barrier function.

In contrast to other studies, this study did not find any association between TEWL and gestational age. However, most differences in TEWL between full term and preterm infants in these studies were found in infants with gestational age less than 32 weeks, of which there were fewer than 40 in our study. As such, we may have been unable to detect an association. Other risk factors associated with TEWL were socioeconomic (SES) factors (higher levels of SES were associated with higher values of TEWL) and sex (being female was associated with lower values of TEWL). One reason why infants of higher SES families might have higher TEWL values is perhaps those infants were wearing more clothes or wrapped in more blankets before the measurements were taken than infants who were of lower SES status. This could cause increased sweating, leading to higher TEWL measurements.

Infants' skin pH in this population decreased throughout the neonatal period, with the greatest decrease occurring during the first week. This supports findings from other studies. (Visscher, Chatterjee et al. 2000; Yosipovitch, Maayan-Metzger et al. 2000) Other risk factors statistically significantly associated with skin pH in this population were being low birthweight (associated with higher values of skin pH), temperature (increasing temperature associated with decreases in skin pH), and humidity (increasing humidity associated with increases in skin pH). The association we found between skin pH and birthweight is in contrast to the previous findings by Green et al. which found no association (Green, Carol et al. 1968) Differences in study design could account for the differences in results however, as all infants in the study by Green et al. were in incubators from birth and were not removed for the purposes of the skin pH measurements. The differing directions of associations between skin pH and temperature and humidity are interesting. Perhaps humidity plays a larger role in sweat response in infants in this setting and the increase in skin pH with increasing humidity could be a result of measuring the pH of sweat on the skin which can result in higher skin pH measurements. (Herrmann and Mandol 1955; Parra and Paye 2003) Decreasing skin pH, indicating improved barrier function, with increases in temperature could be due to the same mechanism as discussed previously for the relationship between TEWL and temperature, increasing blood flow to the skin leading to improved maturation and repair of the skin barrier.

This study also evaluated how stratum corneum protein concentrations removed using skin discs change throughout the first month of life. We found protein concentrations decreased over the first week, followed by a slight increase over the rest of the neonatal period. This decrease in protein concentration indicates the maturation of the stratum corneum over the first week of life. (Berthaud and Boncheva 2011; Myer and Maibach 2013) The slight increase after the first week could be indicative of the regeneration of the stratum corneum. (Chiou and Blume-Peytavi 2004) Other risk factors statistically significantly associated with stratum corneum protein concentrations were: time between measurement and last massage (with greater time since last massage associated with higher levels of protein), SES status (higher SES status associated with lower protein concentrations), and temperature and humidity (increases associated with decreases in protein concentration). Greater time since last massage could result in increases in protein concentration, because the massage may remove the layer of protein that would normally be removed using the skin discs. In addition, the oil may affect whether protein can be removed, as the skin may be more moisturized after recent oil massage. Temperature and humidity may also affect the amount of moisture on the skin creating a barrier between the skin and the disc, which could be the reason for decreases in protein concentration removed by the skin discs with increasing temperature.

Erythema and rash are measures of skin irritation. In this population, erythema scores worsened (increased) over the first 3 days of life, leveled off until about day 14, and then improved (decreased) over the final two weeks. Rash scores worsened over the first two weeks, then improved over the second two weeks. This is in contrast to data from other studies that only found rash in a diapered region due to fecal contact and did not find rash in the chest region. (Visscher, Odio et al. 2009) Smaller infants (those who were of low birthweight or preterm) were associated with lower erythema and rash scores than larger infants. This may be due to the fact that preterm infants have less developed eccrine involvement, resulting in lower prevalence of miliaria, a common rash in newborns caused by retention of sweat. (Foster, Hey et al. 1969; Zuniga and Nguyen 2013) The increase in miliaria was also likely the reason temperature and humidity were associated with increases in rash scores.

Effect of Oil Group on Skin Integrity and Barrier Function

Studies in mice models have shown there might be differences in skin integrity after application of various oils and there have been several studies that have examined the effects of emollients on skin integrity measures comparing emollient treated skin with no emollient treatment. (Darmstadt, Mao-Qiang et al. 2002; Darmstadt, Badrawi et al. 2004; Visscher, Odio et al. 2009) We extend these observations by directly examining, in a community setting, effects on skin integrity measurements when comparing neonates massaged with mustard seed oil to neonates massaged with sunflower seed oil. These data provide some evidence that different types of oil may effect skin integrity measurements when infants are massaged regularly with sunflower oil compared with mustard seed oil. Skin pH measures in the sunflower oil group decreased at a faster rate than in the mustard oil group, indicating a more quickly forming acid mantle, which is important in order for the skin to maintain bacteriological, chemical and mechanical resistance. (Schmid-Wendtner and Korting 2006) In addition, although TEWL measures increased throughout the neonatal period, they increased at a slower rate in the sunflower oil group than in the mustard oil group, which was more pronounced in preterm infants. This could be another indication of a more rapidly improving skin barrier in the sunflower oil group, although both groups in this population had higher TEWL measures than those in other studies. (Visscher, Odio et al. 2009; Kelleher, O'Carroll et al. 2013) There was little evidence that skin condition scores or stratum corneum protein concentrations were different between the two intervention groups.

Effect of Oil Group on Nutritional Status

These data do not provide evidence that full body oil massage with sunflower oil improves neonates' nutritional status when compared with full body oil massage with mustard seed oil. This study found that preterm infants in the sunflower oil group had statistically significantly slower weight gain after day 3 than preterm infants in the mustard oil group. These results differ from those of other studies, which have found improved weight and/or length gain in infants massaged with oils high in linoleic acid. (Arora, Kumar et al. 2005; Vaivre-Douret, Oriot et al. 2009; Fallah, Akhavan Karbasi et al. 2013; Kumar, Upadhyay et al. 2013) However, there were significant differences between the study designs for those studies and our study. Those were all hospital-based studies with study populations who were less than 35 weeks of age or of very low birthweight and as such were generally more vulnerable than our study population. There also may have been differences in the way in which the infants were being fed, as infants in our study population are almost exclusively breastfed. In addition, those studies all used a standardized massage technique using moderate pressure for a prescribed amount of time (usually around 10 minutes two or three times a day). In contrast, our study was community-based with massages performed by mothers or caretakers and done in the traditional (i.e. vigorous) way. These differences in study design could have led to differences in outcomes as improvements in nutritional status in neonates massaged with oils high in linoleic acid may only occur in either very low birthweight or very premature infants, who were unlikely to survive in our setting in rural Nepal. It is uncertain as to what may have led to the slower weight gain in preterm infants in the sunflower oil group, however this small difference in rate of weight gain (about 3 grams less per day) is unlikely to be clinically significant.

This study only compared the differences in nutritional status between infants massaged with an improved oil (sunflower oil) and the traditional oil (mustard oil), therefore, the beneficial nutritional effects of oil massage found in other studies, could be due to massage alone, or massage with any type of oil, and not to the type of oil used. Several studies have shown increased weight gain, less stress behaviors, lower salivary cortisol levels, and increased vagal activity in infants who were massaged without oil when compared with infants who were not massaged. (Field, Schanberg et al. 1996; Ang, Lua et al. 2012; Wang, He et al. 2013) Increased vagal activity, which increases gastric motility, may lead to an increase in food absorption hormones such as insulin and may be one of the underlying mechanisms for greater weight gain in massaged infants. (Field, Diego et al. 2011) An increase in insulin-like growth factor-1 (IGF-1) through an independent pathway unrelated to vagal activity was also related to weight gain perhaps because of fomenting muscle and bone development. (Field, Diego et al. 2011) Also, the type of massage may affect a neonates' nutritional status. Although moderate pressure massage has shown to decrease stress behavior and cortisol levels and increase weight gain compared to no massage, the mode in which massage of infants is conducted in this population is quite vigorous, many times leaving infants stressed and agitated, possibly leading to an increase in cortisol levels, which may influence weight gain. (Field, Diego et al. 2010)

Strengths and Limitations

This study had several strengths. This is the largest study that has investigated skin barrier integrity in infants over the first month of life using a composite measure of visual scoring and more traditional methods (TEWL, skin pH), an approach recommended at a recent expert meeting on emollients. (Expert Emollients Meeting, Bill and Melinda Gates Foundation, December 14, 2012) In addition, although other studies have compared the effect on skin barrier of different oils using a mouse model (Darmstadt, Mao-Qiang et al. 2002), our study expanded on this work, investigating the effects on skin barrier function when comparing mustard and sunflower seed oil in humans. At the same expert meeting, there was a consensus that mouse model work could not be extrapolated to humans and that future work was needed in this area. (Expert Emollients Meeting, Bill and Melinda Gates Foundation, December 14, 2012)

Another strength was our large sample size with an oversampling of preterm infants, which enabled us to detect even small differences between oil groups even when stratified by term status. This study was also conducted in a community “real world” setting where women routinely perform neonatal oil massage using locally available vegetable oils, instead of in a hospital where many other studies examining these relationships have been conducted. In addition, the cluster-randomized design minimized crossover contamination between the two oil groups and helped to avoid selection bias and improve comparability between groups.

This study however, did have some limitations. This study did not include a control group of infants who did not receive massage or infants who received massage without oil. Therefore we were only able to compare the effects of oil massage with the traditional (mustard) and improved (sunflower) oils. We could not make any conclusions on whether the massage itself had any impact on skin integrity or nutritional status in this community. Also, because the measurements were made at home in this rural community, it was sometimes difficult to follow the recommended measurement protocols for the instruments. For example, TEWL and skin pH measures could not be taken in the recommended climate controlled environment (20-22°C and <50% relative humidity). (Rogeries and Group 2001; Parra and Paye 2003) For TEWL measures however, we did follow guidance in taking measurements in non-clinical settings. (du Plessis, Stefaniak et al. 2013) Estimations of gestational age may also have been inaccurate as we used recalled date of last menstrual period (LMP) in order to determine gestational age. This method has been shown to be less accurate than methods using ultrasound. (Rosenberg, Ahmed et al. 2009; Jehan, Zaidi et al. 2010) This inaccuracy may have resulted in misclassification of whether an infant was preterm or full term. However, this misclassification was likely minimal in our study, as LMPs were collected as soon as a pregnancy was identified (following-up women every 5 weeks), so recall bias was likely minimal.

Further Research and Conclusion

Future research using data collected during this study will be conducted on other possible biological mechanisms, which may be affected by neonatal full body oil massage with mustard oil or sunflower oil. These include differences in the effects of the different oils on bacterial colonization of the skin and whether there are differences in immune status and function. In addition, risk factors for bacterial colonization and immune function will be examined in this population in order to better target groups of infants who may be at higher risk of poor immune function or colonization of certain bacteria. Whether the different oil groups have any effect on infants' nutritional status after 6 months will also be explored.

Other future analyses that will be completed using these data are to analyze whether there are any correlations between the different skin integrity measurements (TEWL, skin pH, stratum corneum protein concentration, and skin condition). In addition, the relationships between all of the biological mechanisms studied (skin integrity, bacterial colonization, nutritional status, and immune function) will be examined to determine how they might influence each other. Also, any relationships between morbidity (using data from the NOMS parent trial) and the biological mechanisms will be investigated. Analyses will also be performed limiting our preterm group to those <34 weeks in order to determine if there are effects of the oils on nutritional status and skin integrity on infants who are more premature.

In addition to research on whether there are differences in biological mechanisms between infants massaged with mustard and sunflower oils, research should be conducted on whether there are any differences in biological mechanisms in infants who receive emollient massage, those who receive massage without emollients, and those who receive no massage. It is also important to assess if differences exist in these biological mechanisms in infants who are massaged differently (i.e. vigorous versus gentle massage, or massage with moderate pressure). Although these studies would be difficult to conduct in this population due to the universal cultural practice of neonatal oil massage, these types of studies could possibly be conducted in a smaller group of infants in an urban area where mothers may be more willing to accept behavioral change interventions. (Mullany, Darmstadt et al. 2005) Another possibility is to conduct this type of study in a population of older infants in this rural community, although such an approach would preclude examining differences in skin maturation between different massage approaches.

Since time from last massage was significantly associated with changes in stratum corneum protein concentration, it is also important to perform some controlled experiments in order to further examine the effect that oil massage may have on skin barrier. These experiments should examine variables related to massage, such as the effects of time of oil exposure on protein concentrations, the amount the oil is rubbed in, the pressure used when rubbing in the oil, rubbing with or without oil, etc. These types of experiments could be done on the adult forearm model. (Marty Visscher, personal communication)

Emollient therapy research to improve neonatal survival is a global priority. (Lawn, Zupan et al. 2006) Although millions of babies in South Asia are massaged with mustard oil each year, it is unknown whether substituting sunflower seed oil changes any biological mechanisms that may improve neonatal health. The nearly universal practice of neonatal oil massage in South Asia routinely exposes neonates to the effects of mustard oil massage. Determining how and if certain biological mechanisms are different in infants massaged with sunflower oil will help in the characterization of the modes through which sunflower oil massage may improve neonatal health, and allow for direct comparison of functional indicators across public health outcomes including neonatal skin infection, clinical signs of sepsis, and mortality. These data indicate there may be differences in skin integrity measures in infants massaged with sunflower oil compared with those massaged with mustard seed oil and also discussed some possible risk factors associated with skin integrity in this population in rural southern Nepal. In addition, these data indicated that sunflower oil massage did not improve nutritional status in neonates when compared with mustard oil massage. These results could be applicable to similar populations in northern India, Pakistan, and northwestern Bangladesh, as well as the Terai region in southern Nepal, as they share cultural, social, and economic characteristics. It is important to have a better understanding of the underlying mechanisms of how and why emollient therapy can improve neonatal health outcomes in low-resource settings in order to optimize any health benefits of the oils. Such data are necessary to refine the timing, frequency, and mode of administration in future scaled up programmatic promotion of sunflower oil, a low cost intervention with the potential to substantially improve survival of newborns across South Asia.

Chapter 7 References

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Appendix A Epidemiology and Risk Factors Associated with Left Arm and Right Leg Skin Condition

Table A-1: Descriptive Characteristics of Left Arm and Right Leg Skin Condition

Visit	Measure (Mean (SD))			
	Left Arm Erythema Score	Left Arm Rash Score	Right Leg Erythema Score	Right Leg Rash Score
1	0.67 (0.70)	0.17 (0.46)	0.70 (0.72)	0.15 (0.42)
3	0.90 (0.62)	0.69 (0.78)	0.89 (0.67)	0.54 (0.71)
7	0.96 (0.59)	1.02 (0.87)	0.93 (0.64)	0.76 (0.80)
14	1.01 (0.61)	1.27 (0.88)	0.94 (0.62)	1.06 (0.80)
28	0.67 (0.57)	0.78 (0.74)	0.70 (0.64)	0.77 (0.76)

Table A-2: Left Arm Erythema Score and Infant Characteristics Bivariate Analyses

Predictor Variable	Left Arm Erythema Score			
	Coefficient	SE	p-value	95% CI
Female	0.0046	0.033	0.888	-0.059-0.069
Preterm (<37 wks)	-0.023	0.033	0.486	-0.088-0.042
Gestational Age[a]				
32-36 wks	-0.0027	0.035	0.939	-0.070-0.065
<32 wks	-0.15	0.071	0.036	-0.29- -0.0096
Gestational Age (cont.) (wks)	0.0025	0.0046	0.588	-0.0065-0.011
Low birthweight (<2500g)	-0.079	0.034	0.019	-0.14- -0.013
Birthweight (g) (cont.)	0.00015	0.000034	<0.001	0.000086-0.00022
SGA status[b]				
SGA 3-10%	0.017	0.043	0.694	-0.067-0.10
SGA 3%	-0.11	0.040	0.005	-0.19- -0.035
Breastfed since birth	-0.013	0.046	0.775	-0.10-0.077
Hours before breastfeeding initiation[c]				
1-2 hrs	0.013	0.040	0.748	-0.066-0.092
3-4 hrs	-0.022	0.077	0.769	-0.17-0.13
>=5 hrs	0.054	0.077	0.488	-0.098-0.21
Infant received colostrum	0.045	0.059	0.439	-0.069-0.16
Avg # of massages per day during first week of life	-0.019	0.012	0.111	-0.042-0.0044
Time from visit to last massage[d]				
30-59 min	-0.042	0.036	0.234	-0.11-0.027
60-119 min	-0.046	0.034	0.172	-0.11-0.020
120-179 min	0.0022	0.039	0.954	-0.074-0.078
>180 min	0.013	0.033	0.703	-0.05-0.078
Infant's age days 0 to 3	0.111	0.013	<0.001	0.085-0.14
Infant's age days 4 to 14	0.010	0.003	<0.001	0.0048-0.016
Infant's age days 15 to 28	-0.025	0.002	<0.001	-0.30- -0.021

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 minutes

Table A-3: Left Arm Erythema Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Left Arm Erythema Score			
	Coefficient	SE	p-value	95% CI
Madeshi	-0.13	0.064	0.042	-0.26- -0.0046
Mother literate	-0.023	0.036	0.528	-0.094-0.048
Father literate	0.012	0.033	0.715	-0.052-0.076
Maternal Education[a]				
1-5 yrs	0.030	0.057	0.606	-0.083-0.14
6-10 yrs	-0.046	0.047	0.323	-0.14-0.046
>10 yrs	-0.069	0.082	0.394	-0.23-0.090
Paternal Education[a]				
1-5 yrs	0.047	0.049	0.334	-0.048-0.14
6-10 yrs	0.010	0.038	0.785	-0.063-0.084
>10 yrs	-0.073	0.070	0.293	-0.21-0.064
Household Assets[b, §]				
2-5 assets	-0.0035	0.067	0.959	-0.13-0.13
6-10 assets	-0.031	0.067	0.646	-0.16-0.10
>10 assets	-0.04	0.12	0.731	-0.20-0.28
Household has electricity	-0.099	0.033	0.003	-0.16- -0.033
Maternal Age[c]				
18-24 yrs	0.13	0.050	0.008	0.035-0.23
25-29 yrs	0.14	0.057	0.011	0.034-0.26
30-34 yrs	0.12	0.082	0.147	-0.041-0.28
>=35 yrs	0.057	0.10	0.588	-0.15-0.26
Gravidity[d]				
1-2	0.069	0.040	0.082	-0.0088-0.15
>=3	-0.064	0.045	0.152	-0.024-0.15
Parity[d]				
1-2	0.045	0.040	0.257	-0.033-0.12
>=3	0.048	0.043	0.265	-0.036-0.13
Antenatal Care Visits[d]				
1-2 ANC visits	0.074	0.041	0.068	-0.0055-0.15
>=3 ANC visits	0.080	0.041	0.051	-0.00019-0.16
Delivery at facility	-0.096	0.036	0.008	-0.17- -0.025
Length of labor[e]				
5-14 hrs	-0.021	0.035	0.548	-0.089-0.047
>=15 hrs	-0.090	0.054	0.097	-0.20-0.016

Table A-3: Left Arm Erythema Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses (continued)

Predictor Variable	Left Arm Erythema Score			
	Coefficient	SE	p-value	95% CI
Complications during delivery	-0.040	0.043	0.350	-0.12-0.044
Skilled assistant at delivery	-0.10	0.035	0.005	-0.17- -0.031

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table A-4: Left Arm Erythema Score and Environmental Characteristics Bivariate Analyses

Predictor Variable	Left Arm Erythema Score			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	-0.012	0.0033	<0.001	-0.018- -0.0054
Relative humidity (cont.) (%)	0.0020	0.0011	0.071	-0.00017-0.0041
Relative humidity (%)[a]				
40-59%	-0.084	0.12	0.485	-0.32-0.15
60-79%	-0.0017	0.12	0.988	-0.23-0.22
>80%	-0.018	0.11	0.874	-0.24-0.21
Heat index (°C)	-0.0054	0.0016	0.001	-0.0085- -0.0022

a: Reference group, <40%

Table A-5: Left Arm Erythema Score Multivariate Model

Predictor Variable	Left Arm Erythema Score			
	Coefficient	SE	p-value	95% CI
Gestational age[a]				
32-36 wks	0.025	0.034	0.465	-0.043-0.093
<32 wks	-0.078	0.071	0.289	-0.22-0.064
Female	0.017	0.033	0.603	-0.047-0.081
Madeshi	-0.14	0.062	0.023	-0.27- -0.019
Birthweight (g) (cont.)	0.00014	0.000037	<0.001	0.000067-0.00021
Household has electricity	-0.098	0.033	0.003	-0.16- -0.033
Maternal Age[b]				
18-24 yrs	0.11	0.049	0.029	0.011-0.20
25-29 yrs	0.071	0.057	0.213	-0.041-0.18
30-34 yrs	0.065	0.081	0.425	-0.094-0.22
>= 35 yrs	0.0092	0.10	0.928	-0.19-0.21
Facility delivery	-0.083	0.036	0.022	-0.15- -0.0046
Temperature (°C)	-0.010	0.0034	0.003	-0.017- -0.0036
Relative humidity (%)	0.00018	0.0011	0.874	-0.0020-0.0023
Infant's age days 0 to 3	0.11	0.013	<0.001	0.080-0.13
Infant's age days 4 to 14	0.011	0.0029	<0.001	0.0055-0.017
Infant's age days 15 to 28	-0.026	0.0023	<0.001	-0.031- -0.022

a: Reference group, ≤37 wks; b: Reference group, <18 years

Table A-6: Left Arm Rash Score and Infant Characteristics Bivariate Analyses

Predictor Variable	Left Arm Rash Score			
	Coefficient	SE	p-value	95% CI
Female	-0.0020	0.038	0.959	-0.077-0.073
Preterm (<37 wks)	-0.11	0.039	0.003	-0.19- -0.038
Gestational Age[a]				
32-36 wks	-0.098	0.040	0.015	-0.18- -0.019
<32 wks	-0.22	0.083	0.009	-0.38- -0.053
Gestational Age (cont.) (wks)	0.017	0.0053	0.001	0.0070-0.028
Low birthweight (<2500g)	-0.23	0.039	<0.001	-0.31- -0.16
Birthweight (g) (cont.)	0.00032	0.000039	<0.001	0.00024-0.00040
SGA status[b]				
SGA 3-10%	0.022	0.059	0.661	-0.076-0.12
SGA 3%	-0.16	0.047	0.001	-0.25- -0.069
Breastfed since birth	0.0061	0.054	0.909	-0.090-0.11
Hours before breastfeeding initiation[c]				
1-2 hrs	0.0051	0.048	0.915	-0.088-0.098
3-4 hrs	-0.093	0.090	0.299	-0.27-0.083
>=5 hrs	-0.041	0.091	0.649	-0.22-0.14
Infant received colostrum	0.052	0.069	0.452	-0.084-0.19
Avg # of massages per day during first week of life	-0.041	0.014	0.003	-0.068- -0.014
Time from visit to last massage[d]				
30-59 min	-0.000063	0.049	0.999	-0.097-0.097
60-119 min	0.0094	0.047	0.839	-0.082-0.10
120-179 min	0.076	0.054	0.156	-0.029-0.18
>180 min	0.12	0.046	0.007	0.033-0.21
Infant's age days 0 to 14	0.080	0.003	<0.001	0.074-0.085
Infant's age days 15 to 28	-0.045	0.003	<0.001	-0.051- -0.039

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 minutes

Table A-7: Left Arm Rash Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Left Arm Rash Score			
	Coefficient	SE	p-value	95% CI
Madeshi	-0.20	0.074	0.007	-0.34- -0.053
Mother literate	0.015	0.043	0.716	-0.068-0.099
Father literate	0.054	0.038	0.164	-0.022-0.13
Maternal Education[a]				
1-5 yrs	0.040	0.067	0.549	-0.091-0.17
6-10 yrs	-0.0014	0.055	0.980	-0.11-0.11
>10 yrs	-0.0092	0.096	0.923	-0.20-0.18
Paternal Education[a]				
1-5 yrs	0.012	0.051	0.813	-0.088-0.11
6-10 yrs	0.049	0.040	0.215	-0.028-0.13
>10 yrs	0.057	0.074	0.442	-0.088-0.20
Household Assets[b, §]				
2-5 assets	0.023	0.078	0.766	-0.13-0.18
6-10 assets	0.044	0.078	0.572	-0.11-0.20
>10 assets	0.25	0.14	0.082	-0.032-0.53
Household has electricity	0.0021	0.039	0.957	-0.075-0.079
Maternal Age[c]				
18-24 yrs	0.21	0.058	<0.001	0.094-0.32
25-29 yrs	0.23	0.066	0.001	0.097-0.36
30-34 yrs	0.18	0.095	0.060	-0.0076-0.36
>=35 yrs	0.12	0.12	0.327	-0.12-0.36
Gravidity[d]				
1-2	0.15	0.046	0.001	0.061-0.24
>=3	0.16	0.052	0.001	0.063-0.27
Parity[d]				
1-2	0.14	0.046	0.003	0.045-0.23
>=3	0.18	0.050	<0.001	0.078-0.27
Antenatal Care Visits[d]				
1-2 ANC visits	0.062	0.048	0.191	-0.031-0.16
>=3 ANC visits	0.095	0.048	0.050	0.000030-0.19
Delivery at facility	0.042	0.042	0.322	-0.041-0.13
Length of labor[e]				
5-14 hrs	0.017	0.041	0.685	-0.064-0.097
>=15 hrs	-0.12	0.095	0.069	-0.24-0.0091

Table A-7: Left Arm Rash Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses (continued)

Predictor Variable	Left Arm Rash Score			
	Coefficient	SE	p-value	95% CI
Complications during delivery	-0.018	0.050	0.714	-0.12-0.079
Skilled assistant at delivery	0.036	0.041	0.381	-0.044-0.12

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table A-8: Left Arm Rash Score and Environmental Characteristics Bivariate Analyses

Predictor Variable	Left Arm Rash Score			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	0.023	0.0041	<0.001	0.015-0.031
Relative humidity (cont.) (%)	0.0057	0.0014	<0.001	0.0029-0.0084
Relative humidity (%)[a]				
40-59%	-0.024	0.17	0.888	-0.35-0.31
60-79%	0.22	0.16	0.160	-0.089-0.54
>80%	0.27	0.16	0.092	-0.044-0.58
Heat index (°C)	0.013	0.0019	<0.001	0.0094-0.017

a: Reference group, <40%

Table A-9: Left Arm Rash Score Multivariate Model

Predictor Variable	Left Arm Rash Score			
	Coefficient	SE	p-value	95% CI
Preterm (<37 wks)	-0.10	0.036	0.004	-0.17- -0.032
Female	0.012	0.035	0.717	-0.056-0.082
Madeshi	-0.21	0.073	0.004	-0.35- -0.067
Low birthweight (<2500 g)	-0.16	0.038	<0.001	-0.23- -0.082
Avg # of massages per day during first week of life	-0.0072	0.014	0.596	-0.034-0.019
Maternal Age[a]				
18-24 yrs	0.070	0.059	0.240	-0.046-0.19
25-29 yrs	0.027	0.077	0.728	-0.12-0.18
30-34 yrs	-0.025	0.099	0.805	-0.22-0.17
>= 35 yrs	-0.055	0.122	0.654	-0.29-0.18
Parity[b]				
1-2	0.090	0.048	0.060	-0.0036-0.18
>=3	0.18	0.062	0.004	0.055-0.30
Antenatal Care Visits[b]				
1-2 ANC visits	0.050	0.043	0.245	-0.35-0.13
>=3 ANC visits	0.064	0.044	0.151	-0.023-0.15
Temperature (°C)	0.031	0.0039	<0.001	0.023-0.038
Relative humidity (%)	0.0074	0.0013	<0.001	0.0048-0.010
Infant's age days 0 to 14	0.081	0.0029	<0.001	0.075-0.087
Infant's age days 15 to 28	-0.044	0.0029	<0.001	-0.050- -0.039

a: Reference group, <18 years; b: Reference group, none

Table A-10: Right Leg Erythema Score and Infant Characteristics Bivariate Analyses

Predictor Variable	Right Leg Erythema Score			
	Coefficient	SE	p-value	95% CI
Female	0.042	0.035	0.224	-0.026-0.11
Preterm (<37 wks)	0.0058	0.035	0.870	-0.064-0.075
Gestational Age[a]				
32-36 wks	0.017	0.037	0.640	-0.055-0.090
<32 wks	-0.058	0.076	0.439	-0.21-0.090
Gestational Age (cont.) (wks)	-0.0024	0.0049	0.622	-0.012-0.0071
Low birthweight (<2500g)	-0.058	0.036	0.105	-0.13-0.012
Birthweight (g) (cont.)	0.00014	0.000037	<0.001	0.000069-0.00021
SGA status[b]				
SGA 3-10%	0.011	0.046	0.803	-0.078-0.10
SGA 3%	-0.12	0.043	0.007	-0.20- -0.032
Breastfed since birth	-0.055	0.049	0.261	-0.15-0.041
Hours before breastfeeding initiation[c]				
1-2 hrs	0.051	0.040	0.225	-0.032-0.13
3-4 hrs	0.019	0.077	0.808	-0.14-0.18
>=5 hrs	0.11	0.077	0.159	-0.045-0.27
Infant received colostrum	0.040	0.061	0.519	-0.081-0.16
Avg # of massages per day during first week of life	-0.018	0.013	0.151	-0.043-0.0066
Time from visit to last massage[d]				
30-59 min	-0.043	0.037	0.242	-0.12-0.029
60-119 min	-0.059	0.035	0.090	-0.13-0.0092
120-179 min	0.036	0.040	0.367	-0.12-0.043
>180 min	0.018	0.034	0.603	-0.086-0.050
Infant's age days 0 to 3	0.096	0.014	<0.001	0.069-0.12
Infant's age days 4 to 14	0.0042	0.003	0.160	-0.0017-0.010
Infant's age days 15 to 28	-0.018	0.002	<0.001	-0.023- -0.013

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 minutes

Table A-11: Right Leg Erythema Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Right Leg Erythema Score			
	Coefficient	SE	p-value	95% CI
Madeshi	-0.11	0.068	0.105	-0.24-0.023
Mother literate	-0.029	0.039	0.454	-0.10-0.047
Father literate	0.0056	0.035	0.874	-0.063-0.074
Maternal Education[a]				
1-5 yrs	0.020	0.061	0.747	-0.010-0.14
6-10 yrs	-0.046	0.050	0.360	-0.14-0.052
>10 yrs	-0.094	0.086	0.278	-0.26-0.076
Paternal Education[a]				
1-5 yrs	0.038	0.052	0.461	-0.063-0.14
6-10 yrs	0.012	0.040	0.764	-0.066-0.090
>10 yrs	-0.12	0.074	0.110	-0.26-0.027
Household Assets[b, §]				
2-5 assets	-0.032	0.071	0.654	-0.17-0.11
6-10 assets	-0.056	0.071	0.431	-0.20-0.083
>10 assets	0.018	0.13	0.888	-0.24-0.27
Household has electricity	-0.11	0.036	0.002	-0.18- -0.040
Maternal Age[c]				
18-24 yrs	0.15	0.053	0.006	0.041-0.25
25-29 yrs	0.16	0.060	0.009	0.039-0.28
30-34 yrs	0.13	0.087	0.122	-0.036-0.30
>=35 yrs	0.15	0.11	0.175	-0.067-0.37
Gravidity[d]				
1-2	0.058	0.042	0.172	-0.025-0.14
>=3	-0.091	0.047	0.055	-0.0019-0.18
Parity[d]				
1-2	0.034	0.042	0.427	-0.049-0.12
>=3	0.066	0.066	0.151	-0.024-0.16
Antenatal Care Visits[d]				
1-2 ANC visits	0.065	0.043	0.133	-0.020-0.15
>=3 ANC visits	0.041	0.044	0.352	-0.045-0.13
Delivery at facility	-0.16	0.038	<0.001	-0.24- -0.086
Length of labor[e]				
5-14 hrs	-0.052	0.037	0.163	-0.12-0.021
>=15 hrs	-0.107	0.058	0.063	-0.22-0.0058

Table A-11: Right Leg Erythema Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses (continued)

Predictor Variable	Right Leg Erythema Score			
	Coefficient	SE	p-value	95% CI
Complications during delivery	-0.056	0.045	0.214	-0.15-0.032
Skilled assistant at delivery	-0.15	0.037	<0.001	-0.22- -0.081

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table A-12: Right Leg Erythema Score and Environmental Characteristics Bivariate Analyses

Predictor Variable	Right Leg Erythema Score			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	-0.014	0.0035	<0.001	-0.021- -0.0071
Relative humidity (cont.) (%)	0.00093	0.0011	0.414	-0.0013-0.0032
Relative humidity (%)[a]				
40-59%	0.14	0.13	0.260	-0.10-0.39
60-79%	0.13	0.12	0.278	-0.10-0.36
>80%	0.13	0.12	0.264	-0.10-0.37
Heat index (°C)	-0.0064	0.0017	<0.001	-0.0097- -0.0031

a: Reference group, <40%

Table A-13: Right Leg Erythema Score Multivariate Model

Predictor Variable	Right Leg Erythema Score			
	Coefficient	SE	p-value	95% CI
Preterm (<37 weeks)	0.028	0.035	0.430	-0.041-0.096
Female	0.060	0.034	0.080	-0.0071-0.13
Madeshi	-0.14	0.066	0.038	-0.27- -0.0078
Birthweight (g) (cont.)	0.00014	0.000038	<0.001	0.00006-0.00021
Household has electricity	-0.10	0.035	0.004	-0.17- -0.033
Maternal Age[a]				
18-24 yrs	0.13	0.052	0.012	0.029-0.23
25-29 yrs	0.092	0.060	0.129	-0.027-0.21
30-34 yrs	0.085	0.085	0.320	-0.082-0.25
>= 35 yrs	0087	0.11	0.422	-0.12-0.30
Facility delivery	-0.15	0.038	<0.001	-0.22- -0.073
Temperature (°C)	-0.014	0.0036	<0.001	-0.021- -0.0072
Relative humidity (%)	-0.0011	0.0012	0.363	-0.0034-0.0012
Infant's age days 0 to 3	0.089	0.014	<0.001	0.062-0.12
Infant's age days 4 to 14	0.0050	0.0031	0.108	-0.0023-0.014
Infant's age days 15 to 28	-0.019	0.0024	<0.001	-0.023- -0.014

a: Reference group, <18 years

Table A-14: Right Leg Rash Score and Infant Characteristics Bivariate Analyses

Predictor Variable	Right Leg Rash Score			
	Coefficient	SE	p-value	95% CI
Female	-0.0042	0.034	0.903	-0.071-0.063
Preterm (<37 wks)	-0.095	0.035	0.006	-0.16- -0.028
Gestational Age[a]				
32-36 wks	-0.10	0.036	0.005	-0.17- -0.031
<32 wks	-0.061	0.074	0.407	-0.21-0.083
Gestational Age (cont.) (wks)	0.010	0.0048	0.034	0.0075-0.019
Low birthweight (<2500g)	-0.20	0.034	<0.001	-0.27- -0.13
Birthweight (g) (cont.)	0.00027	0.000035	<0.001	0.00020-0.00034
SGA status[b]				
SGA 3-10%	0.021	0.045	0.633	-0.066-0.11
SGA 3%	-0.16	0.041	<0.001	-0.24- -0.081
Breastfed since birth	-0.0083	0.048	0.862	-0.10-0.085
Hours before breastfeeding initiation[c]				
1-2 hrs	0.014	0.042	0.749	-0.069-0.096
3-4 hrs	-0.018	0.080	0.822	-0.17-0.14
>=5 hrs	-0.0090	0.081	0.911	-0.17-0.15
Infant received colostrum	0.014	0.061	0.825	-0.11-0.13
Avg # of massages per day during first week of life	-0.032	0.012	0.010	-0.056- -0.0077
Time from visit to last massage[d]				
30-59 min	-0.055	0.045	0.224	-0.14-0.034
60-119 min	0.032	0.043	0.441	-0.12-0.051
120-179 min	0.040	0.049	0.412	-0.056-0.14
>180 min	0.11	0.042	0.008	0.029-0.19
Infant's age days 0 to 14	0.066	0.003	<0.001	0.060-0.071
Infant's age days 15 to 28	-0.027	0.003	<0.001	-0.0323- -0.022

a: Reference group, ≥37 weeks; b: Reference group, AGA; c: Reference group, <1 hour; d: Reference group, <30 minutes

Table A-15: Right Leg Rash Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses

Predictor Variable	Right Leg Rash Score			
	Coefficient	SE	p-value	95% CI
Madeshi	-0.13	0.066	0.056	-0.26-0.0031
Mother literate	0.0029	0.038	0.939	-0.071-0.077
Father literate	0.011	0.034	0.753	-0.056-0.078
Maternal Education[a]				
1-5 yrs	0.029	0.060	0.630	-0.088-0.15
6-10 yrs	-0.031	0.049	0.524	-0.13-0.065
>10 yrs	0.071	0.085	0.404	-0.096-0.24
Paternal Education[a]				
1-5 yrs	-0.050	0.051	0.324	-0.15-0.049
6-10 yrs	0.017	0.039	0.653	-0.059-0.094
>10 yrs	0.0086	0.073	0.906	-0.13-0.15
Household Assets[b, §]				
2-5 assets	-0.036	0.069	0.610	-0.17-0.10
6-10 assets	-0.0045	0.069	0.948	-0.14-0.13
>10 assets	0.20	0.13	0.110	-0.046-0.45
Household has electricity	0.039	0.035	0.260	-0.029-0.11
Maternal Age[c]				
18-24 yrs	0.14	0.058	0.005	0.043-0.25
25-29 yrs	0.15	0.066	0.021	0.034-0.27
30-34 yrs	0.18	0.095	0.037	0.011-0.34
>=35 yrs	0.17	0.12	0.227	-0.043-0.38
Gravidity[d]				
1-2	0.010	0.041	0.016	0.019-0.18
>=3	0.15	0.046	0.001	0.061-0.24
Parity[d]				
1-2	0.093	0.041	0.023	0.013-0.17
>=3	0.17	0.045	<0.001	0.079-0.25
Antenatal Care Visits[d]				
1-2 ANC visits	0.046	0.042	0.281	-0.037-0.13
>=3 ANC visits	0.045	0.043	0.291	-0.039-0.13
Delivery at facility	0.022	0.038	0.562	-0.052-0.096
Length of labor[e]				
5-14 hrs	0.038	0.036	0.295	-0.033-0.11
>=15 hrs	-0.057	0.057	0.312	-0.17-0.054

Table A-15: Right Leg Rash Score and Maternal, Socioeconomic, and Household Characteristics Bivariate Analyses (continued)

Predictor Variable	Right Leg Rash Score			
	Coefficient	SE	p-value	95% CI
Complications during delivery	-0.018	0.044	0.693	-0.070-0.10
Skilled assistant at delivery	0.020	0.046	0.574	-0.051-0.092

a: Reference group, no education; b: Reference group, 0 or 1 asset(s); c: Reference group, <18 years; d: Reference group, none; e: Reference group, <5 hours; §: HH assets were based on having at least one of the following: electricity, servants, 2nd floor, cattle, goats, bullock carts, bicycles, clocks, radios, TVs, phones, plot of farmable land, plot of other land, family member living & working out of home.

Table A-16: Right Leg Rash Score and Environmental Characteristics Bivariate Analyses

Predictor Variable	Right Leg Rash Score			
	Coefficient	SE	p-value	95% CI
Temperature (°C)	0.016	0.0037	<0.001	0.0092-0.024
Relative humidity (cont.) (%)	0.0045	0.0013	<0.001	0.0020-0.0070
Relative humidity (%)[a]				
40-59%	0.16	0.15	0.304	-0.14-0.46
60-79%	0.30	0.15	0.039	0.016-0.59
>80%	0.36	0.15	0.015	0.070-0.65
Heat index (°C)	0.010	0.0017	<0.001	0.0068-0.014

a: Reference group, <40%

Table A-17: Right Leg Rash Score Multivariate Model

Predictor Variable	Right Leg Rash Score			
	Coefficient	SE	p-value	95% CI
Preterm (<37 wks)	-0.085	0.033	0.010	-0.15- -0.020
Female	0.0028	0.033	0.813	-0.062-0.067
Madeshi	-0.17	0.069	0.028	-0.30- -0.032
Low birthweight (<2500 g)	-0.15	0.035	<0.001	-0.22- -0.085
Avg # of massages per day during first week of life	-0.0032	0.013	0.798	-0.028-0.022
Time from visit to last massage[a]				
30-59 min	-0.084	0.042	0.046	-0.17- -0.0015
60-119 min	-0.093	0.040	0.019	-0.17- -0.015
120-179 min	-0.053	0.046	0.246	-0.14-0.036
>180 min	-0.035	0.040	0.376	-0.11-0.043
Maternal Age[b]				
18-24 yrs	0.028	0.054	0.611	-0.080-0.14
25-29 yrs	-0.059	0.071	0.405	-0.20-0.080
30-34 yrs	-0.042	0.091	0.651	-0.22-0.14
>= 35 yrs	-0.029	0.11	0.798	-0.25-0.19
Parity[c]				
1-2	0.054	0.044	0.219	-0.032-0.14
>=3	0.19	0.057	0.001	0.076-0.30
Temperature (°C)	0.022	0.0036	<0.001	0.015-0.029
Relative humidity (%)	0.0059	0.0013	<0.001	0.0034-0.0083
Infant's age days 0 to 14	0.067	0.0028	<0.001	0.061-0.072
Infant's age days 15 to 28	-0.026	0.0028	<0.001	-0.032- -0.021

a: Reference group, <30 min; b: Reference group, <18 years; c: Reference group, none

Figure A-1: Left Arm Skin Condition Score During Neonatal Period

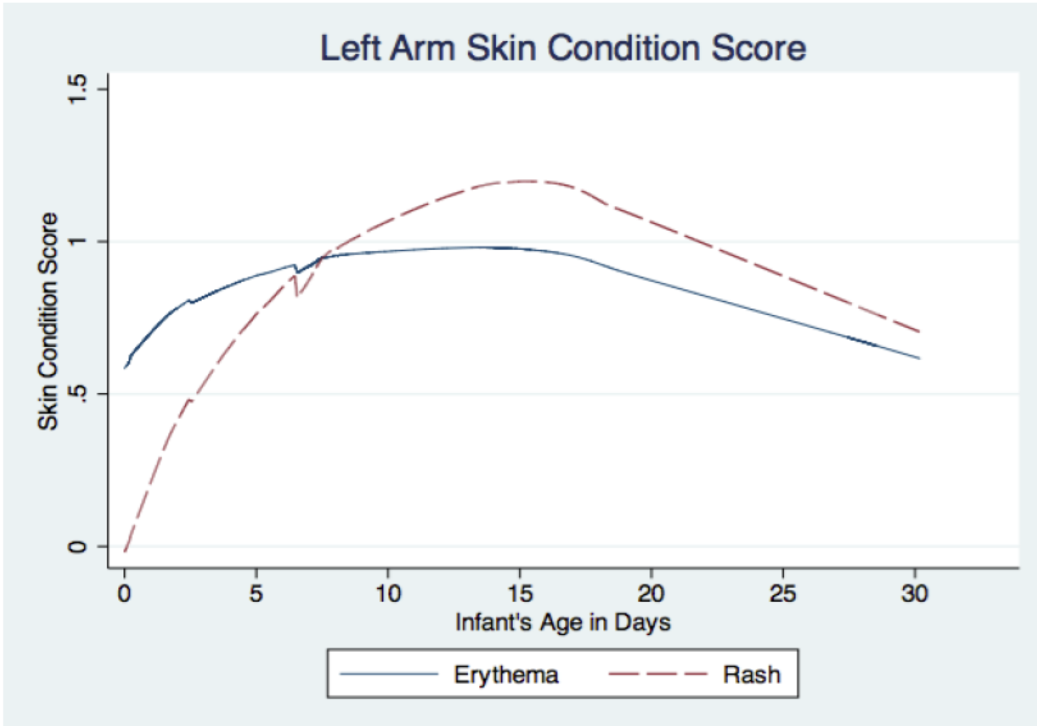
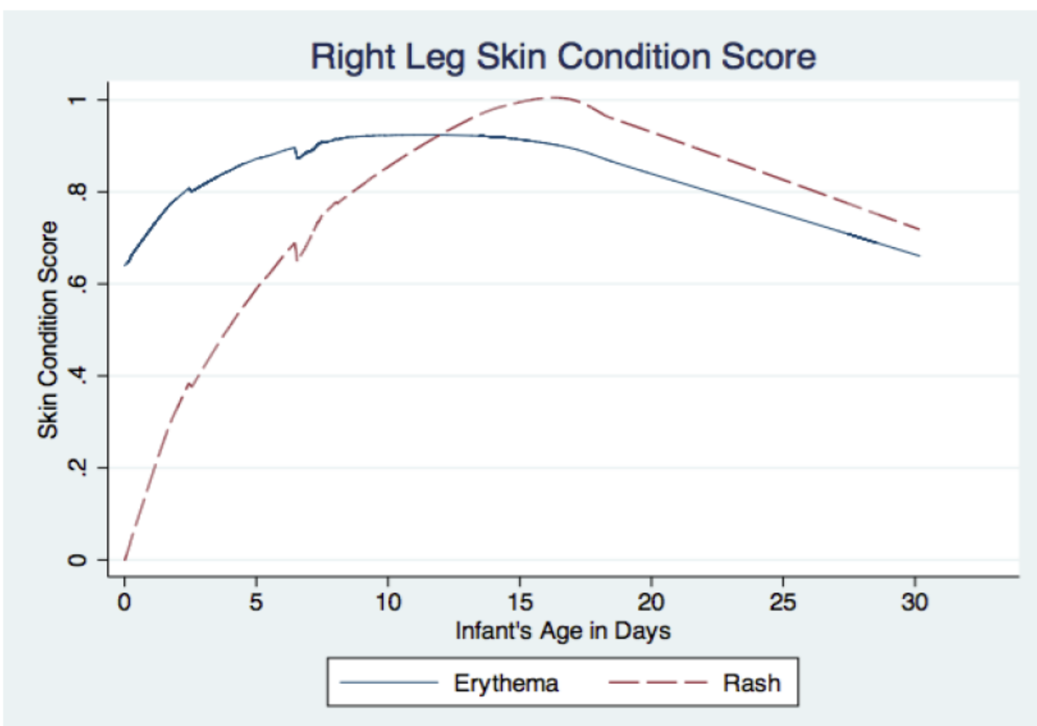


Figure A-2: Right Leg Skin Condition Score During Neonatal Period



Appendix B Effect of Oil Group on Skin Integrity and Barrier Function (Left Arm and Right Leg Skin Condition and Full Term Group for All Measurements)

Table B-1: Left Arm Skin Condition Score by Visit and Intervention Group-Complete Data

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	324	56	0.68	0.70	308	57	0.67	0.70
Rash	324	56	0.21	0.51	308	57	0.14	0.41
Total	324	56	0.89	0.98	308	57	0.81	0.89
Visit 3								
Erythema	315	56	0.92	0.63	303	57	0.89	0.61
Rash	315	56	0.72	0.77	303	57	0.66	0.78
Total	315	56	1.64	1.20	303	57	1.55	1.16
Visit 7								
Erythema	312	56	0.92	0.57	305	57	1.00	0.61
Rash	312	56	1.04	0.85	305	57	1.00	0.90
Total	312	56	1.96	1.24	305	57	2.00	1.31
Visit 14								
Erythema	309	56	1.01	0.62	299	57	1.02	0.60
Rash	309	56	1.29	0.86	299	57	1.26	0.89
Total	309	56	2.30	1.36	299	57	2.27	1.36
Visit 28								
Erythema	270	56	0.69	0.55	262	57	0.66	0.59
Rash	270	56	0.78	0.74	262	57	0.78	0.74
Total	270	56	1.47	1.15	262	57	1.44	1.22

N=number of observations, n=number of clusters, SD=standard deviation

Table B-2: Descriptive Characteristics of Left Arm Skin Condition Score by Visit and Intervention Group-Full Term

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	175	52	0.71	0.70	184	52	0.62	0.66
Rash	175	52	0.27	0.57	184	52	0.17	0.44
Total	175	52	0.98	1.02	184	52	0.78	0.90
Visit 3								
Erythema	169	51	0.94	0.62	180	52	0.89	0.60
Rash	169	51	0.72	0.75	180	52	0.68	0.80
Total	169	51	1.67	1.19	180	52	1.57	1.17
Visit 7								
Erythema	168	51	0.90	0.58	183	52	1.02	0.60
Rash	168	51	1.05	0.87	183	52	1.08	0.88
Total	168	51	1.96	1.28	183	52	2.11	1.29
Visit 14								
Erythema	167	51	1.08	0.64	181	52	1.04	0.59
Rash	167	51	1.43	0.85	181	52	1.35	0.86
Total	167	51	2.51	1.37	181	52	2.39	1.34
Visit 28								
Erythema	144	50	0.73	0.55	155	51	0.65	0.57
Rash	144	50	0.83	0.73	155	51	0.85	0.73
Total	144	50	1.56	1.15	155	51	1.49	1.21

N=number of observations, n=number of clusters, SD=standard deviation

Table B-3: Descriptive Characteristics of Left Arm Skin Condition Score by Visit and Intervention Group-Preterm

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	144	53	0.65	0.71	121	47	0.76	0.75
Rash	144	53	0.15	0.42	121	47	0.10	0.36
Total	144	53	0.80	0.94	121	47	0.85	0.89
Visit 3								
Erythema	142	53	0.89	0.64	120	47	0.90	0.63
Rash	142	53	0.74	0.80	120	47	0.62	0.75
Total	142	53	1.63	1.23	120	47	1.52	1.16
Visit 7								
Erythema	140	53	0.96	0.56	119	47	0.98	0.60
Rash	140	53	1.04	0.82	119	47	0.90	0.93
Total	140	53	1.99	1.19	119	47	1.88	1.30
Visit 14								
Erythema	138	53	0.95	0.59	116	47	0.99	0.61
Rash	138	53	1.14	0.84	116	47	1.13	0.93
Total	138	53	2.09	1.30	116	47	2.12	1.36
Visit 28								
Erythema	123	51	0.64	0.55	105	45	0.67	0.61
Rash	123	51	0.74	0.74	105	45	0.70	0.75
Total	123	51	1.38	1.15	105	45	1.38	1.24

N=number of observations, n=number of clusters, SD=standard deviation

Table B-4: Regression Results of Intervention Group On Left Arm Skin Condition Total Score

	N	n	Coefficient	SE	95% CI
Complete Data Set					
Visit 1	632	113	-0.08	0.08	-0.23-0.07
Visit 3	618	113	-0.09	0.10	-0.28-0.09
Visit 7	617	113	0.03	0.10	-0.17-0.23
Visit 14	608	113	-0.03	0.11	-0.25-0.19
Visit 28	532	113	-0.02	0.11	-0.23-0.20
Complete Neonatal Period	632	113	-0.03	0.07	-0.16-0.10
Early Neonatal Period	632	113	-0.09	0.07	-0.23-0.05
Late Neonatal Period	614	113	0.02	0.09	-0.16-0.20
Full Term					
Visit 1	359	104	-0.20	0.10	-0.40-0.01
Visit 3	349	103	-0.09	0.13	-0.34-0.16
Visit 7	351	103	0.15	0.14	-0.12-0.43
Visit 14	348	103	-0.12	0.15	-0.40-0.17
Visit 28	299	101	-0.07	0.13	-0.34-0.20
Complete Neonatal Period	359	104	-0.05	0.08	-0.21-0.11
Early Neonatal Period	359	104	-0.08	0.09	-0.27-0.10
Late Neonatal Period	350	103	-0.03	0.12	-0.26-0.20
Preterm					
Visit 1	265	100	0.06	0.11	-0.16-0.28
Visit 3	262	100	-0.11	0.15	-0.40-0.18
Visit 7	259	100	-0.12	0.15	-0.42-0.19
Visit 14	254	100	0.02	0.18	-0.33-0.38
Visit 28	228	96	-0.003	0.17	-0.33-0.32
Complete Neonatal Period	265	100	-0.04	0.09	-0.22-0.15
Early Neonatal Period	265	100	-0.10	0.11	-0.31-0.11
Late Neonatal Period	257	100	0.04	0.14	-0.24-0.32

N=number of observations for visits and number of infants for neonatal periods, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE=standard errors, 95% CI=95% confidence interval

Table B-5: Descriptive Characteristics of Right Leg Skin Condition Score by Visit and Intervention Group-Complete Data

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	324	56	0.70	0.72	308	57	0.69	0.72
Rash	324	56	0.18	0.48	308	57	0.12	0.34
Total	324	56	0.89	0.97	308	57	0.81	0.87
Visit 3								
Erythema	315	56	0.89	0.67	303	57	0.89	0.67
Rash	315	56	0.57	0.72	303	57	0.51	0.70
Total	315	56	1.46	1.18	303	57	1.40	1.13
Visit 7								
Erythema	312	56	0.89	0.62	305	57	0.97	0.66
Rash	312	56	0.78	0.81	305	57	0.75	0.79
Total	312	56	1.67	1.20	305	57	1.72	1.19
Visit 14								
Erythema	309	56	0.92	0.63	299	57	0.96	0.61
Rash	309	56	1.05	0.75	299	57	1.07	0.84
Total	309	56	1.96	1.23	299	57	2.03	1.28
Visit 28								
Erythema	270	56	0.72	0.62	262	57	0.68	0.65
Rash	270	56	0.79	0.76	262	57	0.74	0.75
Total	270	56	1.51	1.25	262	57	1.42	1.27

N=number of observations, n=number of clusters, SD=standard deviation

Table B-6: Descriptive Characteristics of Right Leg Skin Condition Score by Visit and Intervention Group-Full Term

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	175	52	0.73	0.72	184	52	0.62	0.67
Rash	175	52	0.23	0.55	184	52	0.14	0.38
Total	175	52	0.96	1.00	184	52	0.77	0.85
Visit 3								
Erythema	169	51	0.88	0.66	180	52	0.88	0.67
Rash	169	51	0.54	0.74	180	52	0.50	0.70
Total	169	51	1.42	1.20	180	52	1.38	1.15
Visit 7								
Erythema	168	51	0.86	0.62	183	52	0.97	0.65
Rash	168	51	0.78	0.79	183	52	0.85	0.80
Total	168	51	1.64	1.20	183	52	1.82	1.21
Visit 14								
Erythema	167	51	0.94	0.62	181	52	1.00	0.61
Rash	167	51	1.13	0.76	181	52	1.14	0.83
Total	167	51	2.06	1.23	181	52	2.15	1.29
Visit 28								
Erythema	144	50	0.76	0.63	155	51	0.67	0.62
Rash	144	50	0.89	0.78	155	51	0.84	0.77
Total	144	50	1.65	1.29	155	51	1.51	1.28

N=number of observations, n=number of clusters, SD=standard deviation

Table B-7: Descriptive Characteristics of Right Leg Skin Condition Score by Visit and Intervention Group-Preterm

	Mustard Oil				Sunflower Oil			
	N	n	Mean	SD	N	n	Mean	SD
Visit 1								
Erythema	144	53	0.68	0.73	121	47	0.80	0.79
Rash	144	53	0.14	0.38	121	47	0.08	0.29
Total	144	53	0.82	0.92	121	47	0.88	0.90
Visit 3								
Erythema	142	53	0.90	0.67	120	47	0.93	0.66
Rash	142	53	0.62	0.71	120	47	0.53	0.71
Total	142	53	1.53	1.16	120	47	1.45	1.12
Visit 7								
Erythema	140	53	0.93	0.62	119	47	0.98	0.66
Rash	140	53	0.79	0.84	119	47	0.61	0.77
Total	140	53	1.71	1.19	119	47	1.59	1.13
Visit 14								
Erythema	138	53	0.91	0.64	116	47	0.92	0.60
Rash	138	53	0.96	0.73	116	47	0.97	0.85
Total	138	53	1.87	1.21	116	47	1.89	1.23
Visit 28								
Erythema	123	51	0.67	0.62	105	45	0.70	0.68
Rash	123	51	0.69	0.73	105	45	0.61	0.71
Total	123	51	1.36	1.21	105	45	1.31	1.26

N=number of observations, n=number of clusters, SD=standard deviation

Table B-8: Regression Results of Intervention Group on Right Leg Skin Condition Total Score

	N	n	Coefficient	SE	95% CI
Complete Data Set					
Visit 1	632	113	-0.08	0.07	-0.22-0.06
Visit 3	618	113	-0.06	0.09	-0.24-0.12
Visit 7	617	113	0.05	0.10	-0.14-0.23
Visit 14	608	113	0.07	0.10	-0.13-0.27
Visit 28	532	113	-0.08	0.11	-0.30-0.13
Complete Neonatal Period	632	113	-0.02	0.06	-0.13-0.10
Early Neonatal Period	632	113	-0.06	0.07	-0.19-0.07
Late Neonatal Period	614	113	0.03	0.08	-0.13-0.19
Full Term					
Visit 1	359	104	-0.19	0.10	-0.38-0.004
Visit 3	349	103	-0.05	0.13	-0.30-0.20
Visit 7	351	103	0.19	0.13	-0.07-0.44
Visit 14	348	103	0.08	0.14	-0.18-0.35
Visit 28	299	101	-0.14	0.15	-0.43-0.15
Complete Neonatal Period	359	104	-0.01	0.08	-0.16-0.14
Early Neonatal Period	359	104	-0.08	0.09	-0.26-0.11
Late Neonatal Period	350	103	0.07	0.11	-0.14-0.28
Preterm					
Visit 1	265	100	0.06	0.11	-0.16-0.28
Visit 3	262	100	-0.07	0.14	-0.35-0.20
Visit 7	259	100	-0.12	0.14	-0.40-0.17
Visit 14	254	100	0.02	0.15	-0.28-0.32
Visit 28	228	96	-0.05	0.17	-0.38-0.27
Complete Neonatal Period	265	100	-0.04	0.09	-0.21-0.14
Early Neonatal Period	265	100	-0.04	0.10	-0.24-0.16
Late Neonatal Period	257	100	-0.05	0.13	-0.30-0.20

N=number of observations for visits and number of infants for neonatal periods, n=number of clusters, Coefficient=regression coefficient for sunflower group, SE=standard errors, 95% CI=95% confidence interval

Figure B-1: TEWL by Infant's Age-Full Term

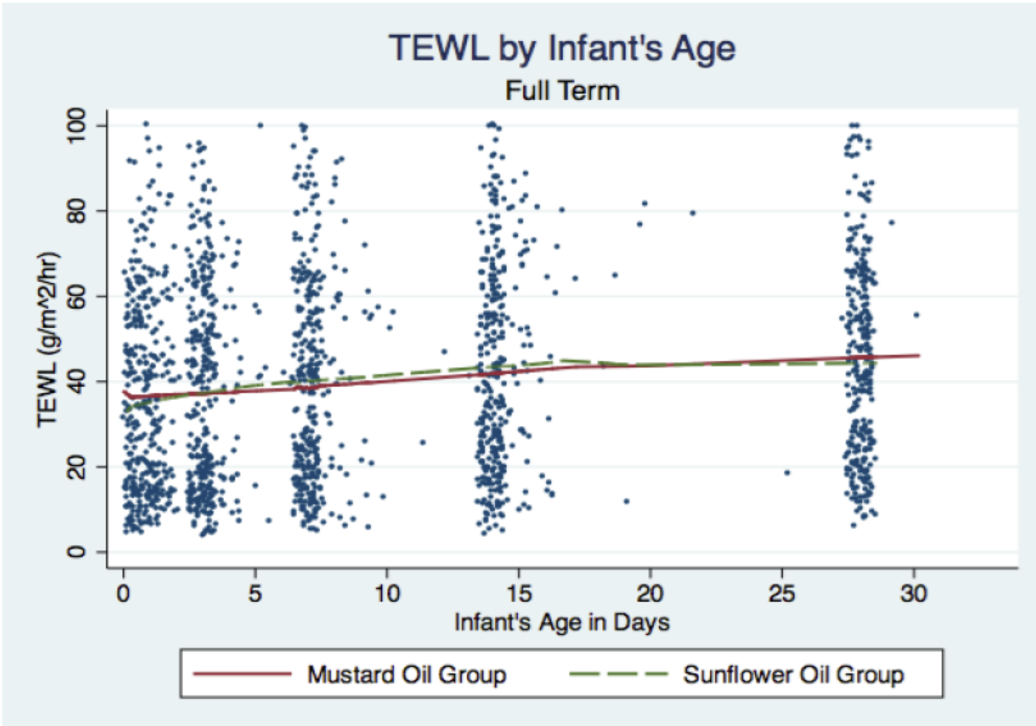


Figure B-2: Skin pH by Infant's Age-Full Term

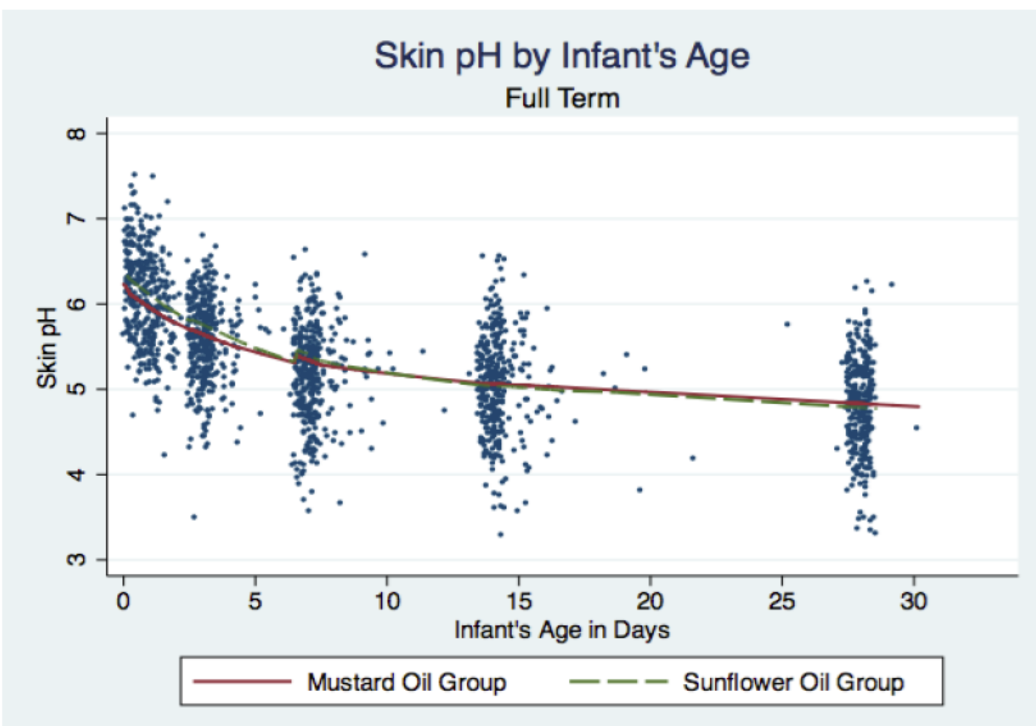


Figure B-3: Skin pH Cox Proportional Hazards Regression Survival Curves-Full Term

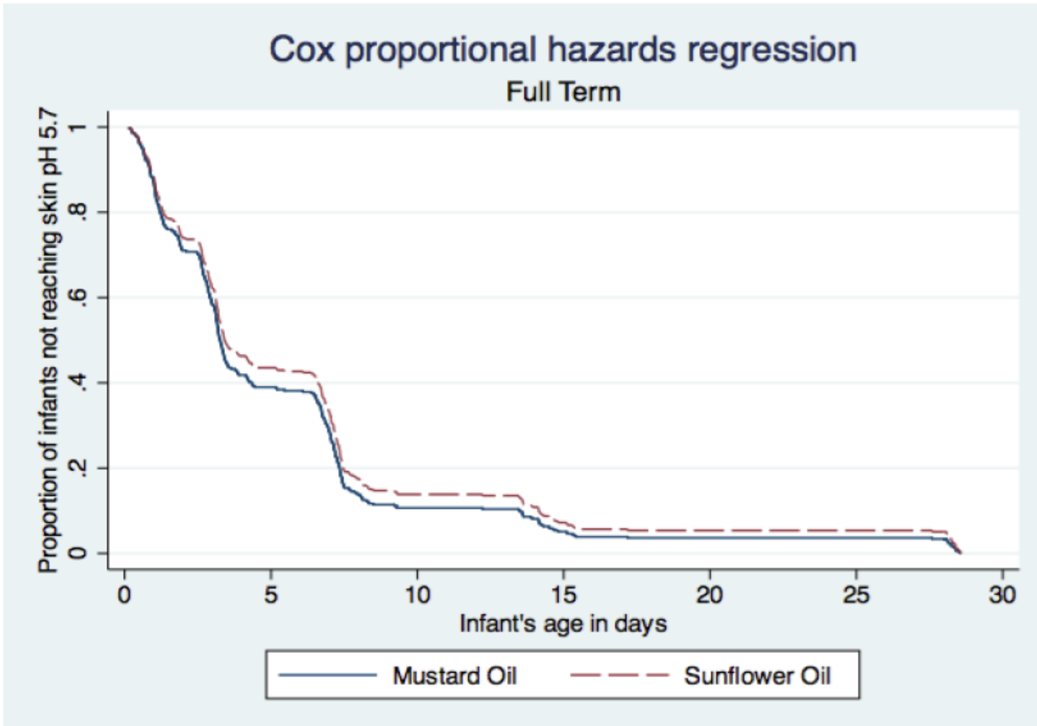


Figure B-4: Stratum Corneum Protein Concentration by Infant's Age-Full Term

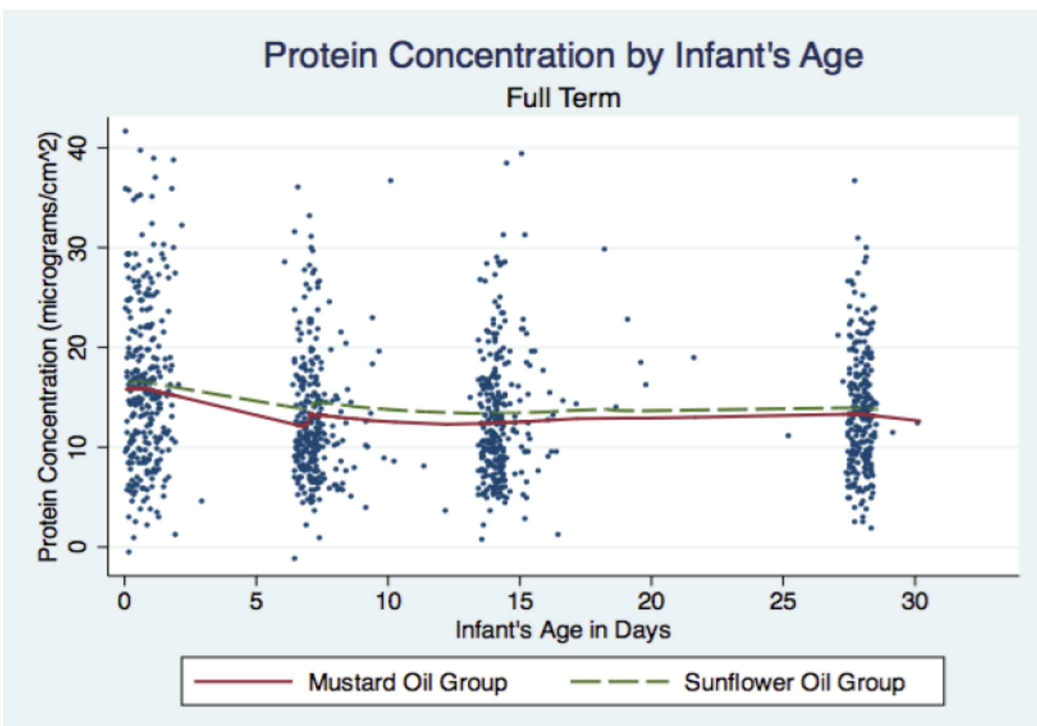


Figure B-5: Chest Skin Condition Total Score by Infant's Age-Full Term

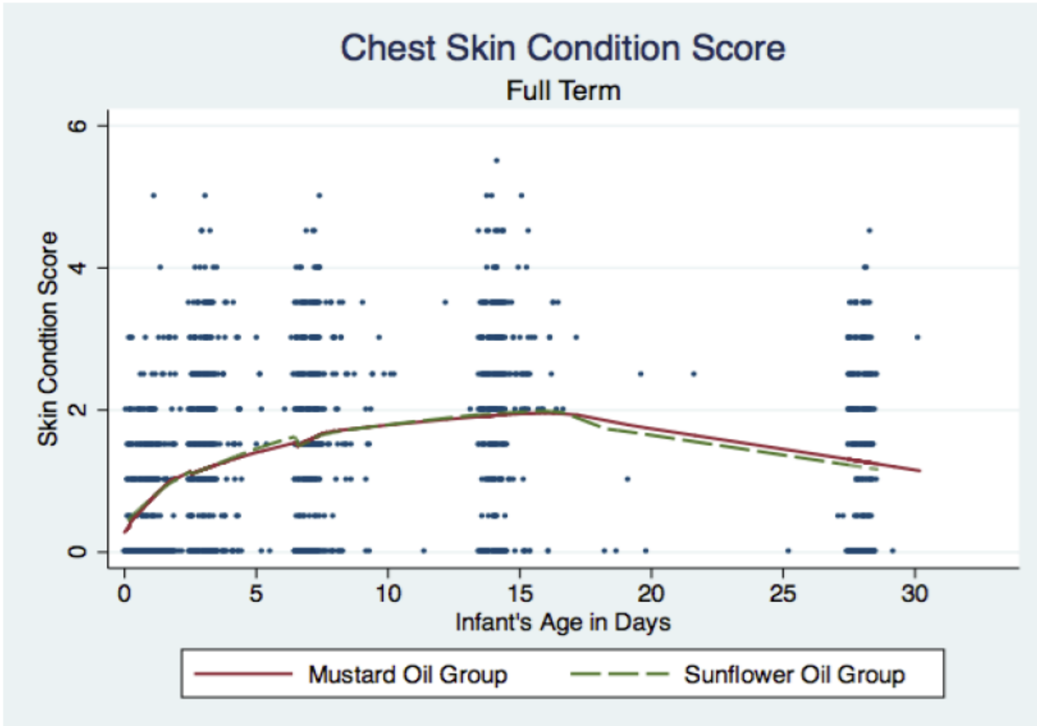


Figure B-6: Left Arm Skin Condition Total Score by Infant's Age-Complete Data

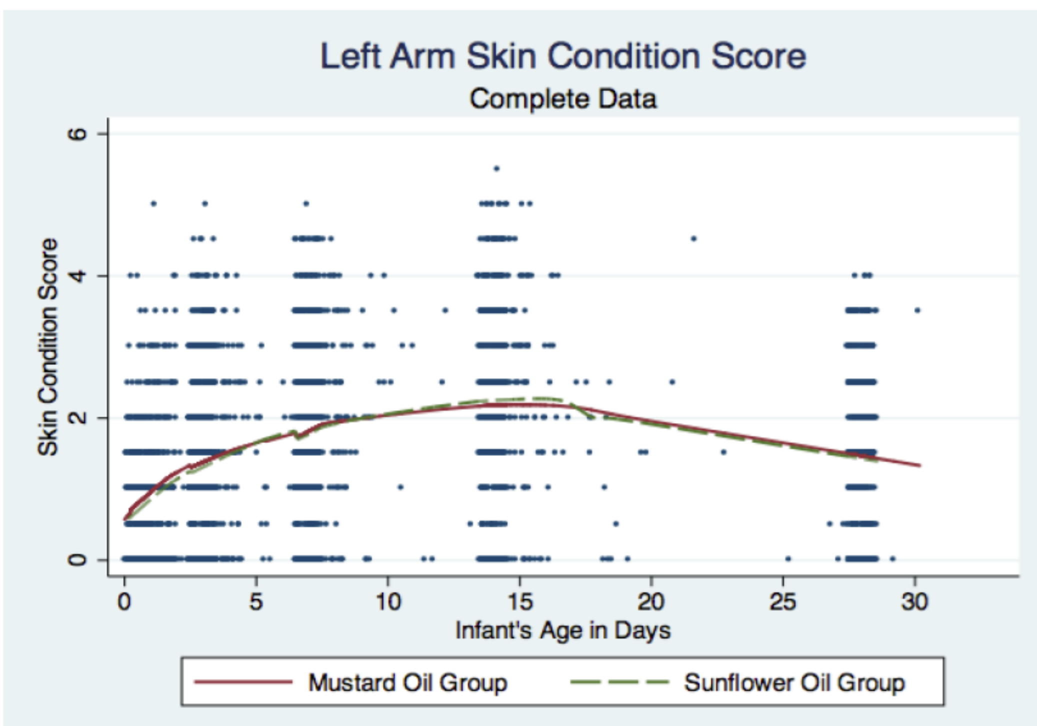


Figure B-7: Left Arm Skin Condition Total Score by Infant's Age-Full Term

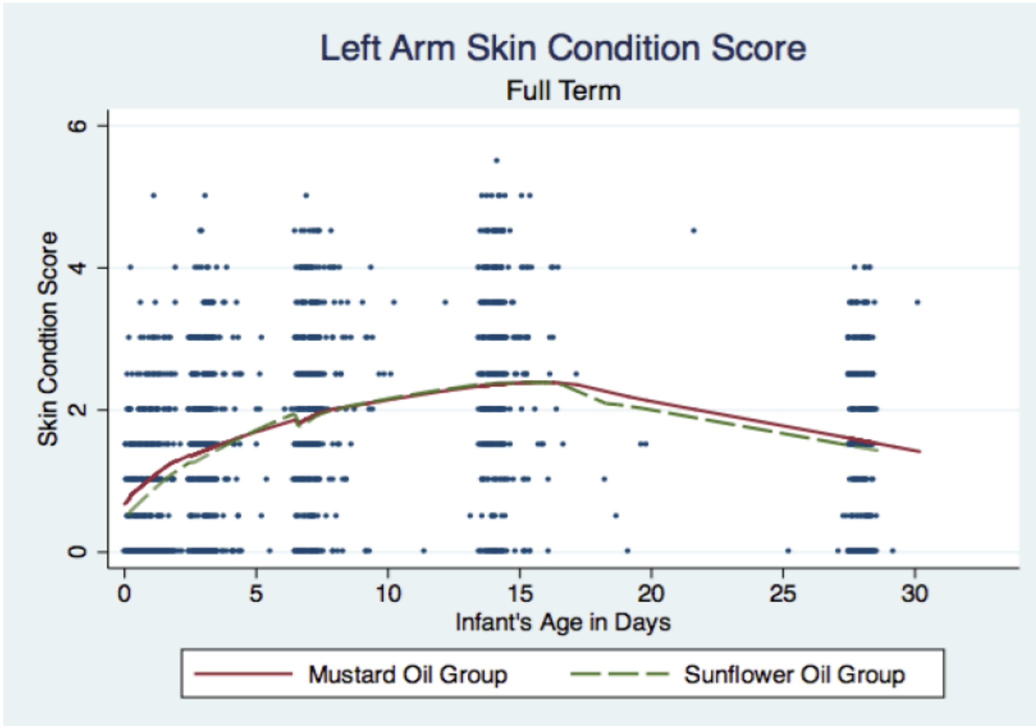


Figure B-8: Left Arm Skin Condition Total Score by Infant's Age-Preterm

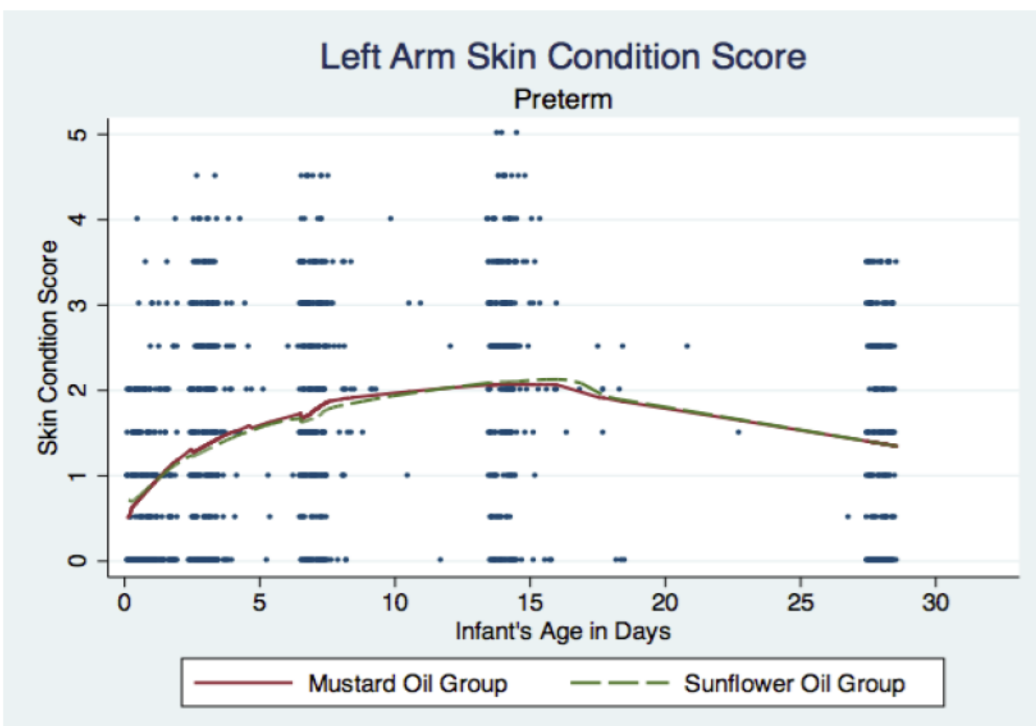


Figure B-9: Left Arm Skin Condition Score by Component and Intervention Group-Complete Data

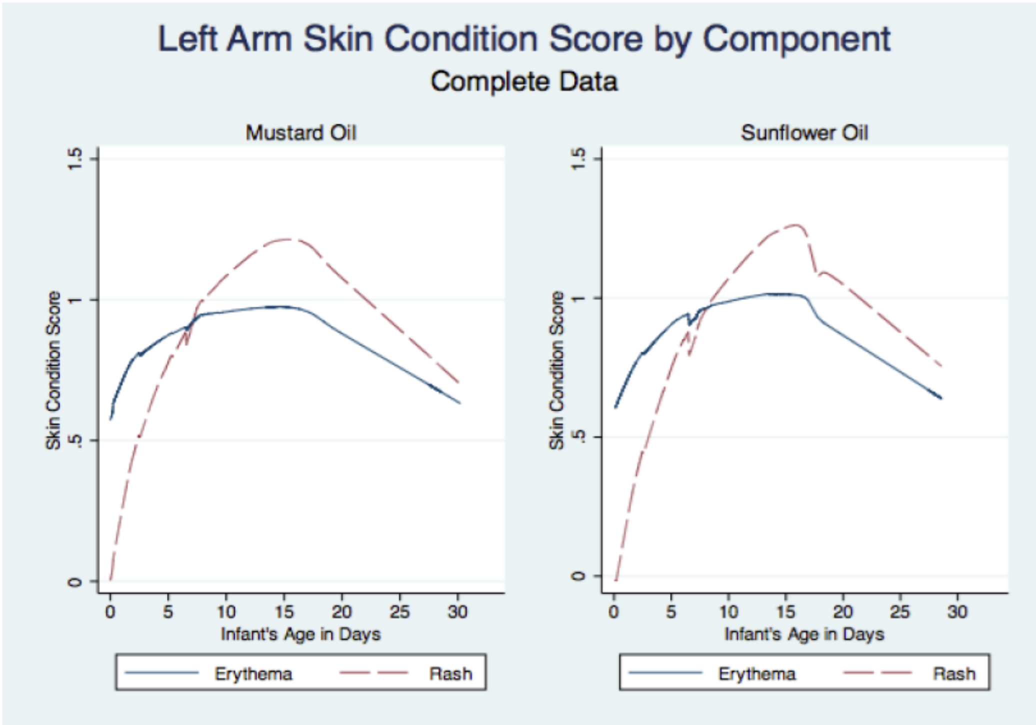


Figure B-10: Right Leg Skin Condition Total Score by Infant's Age-Complete Data

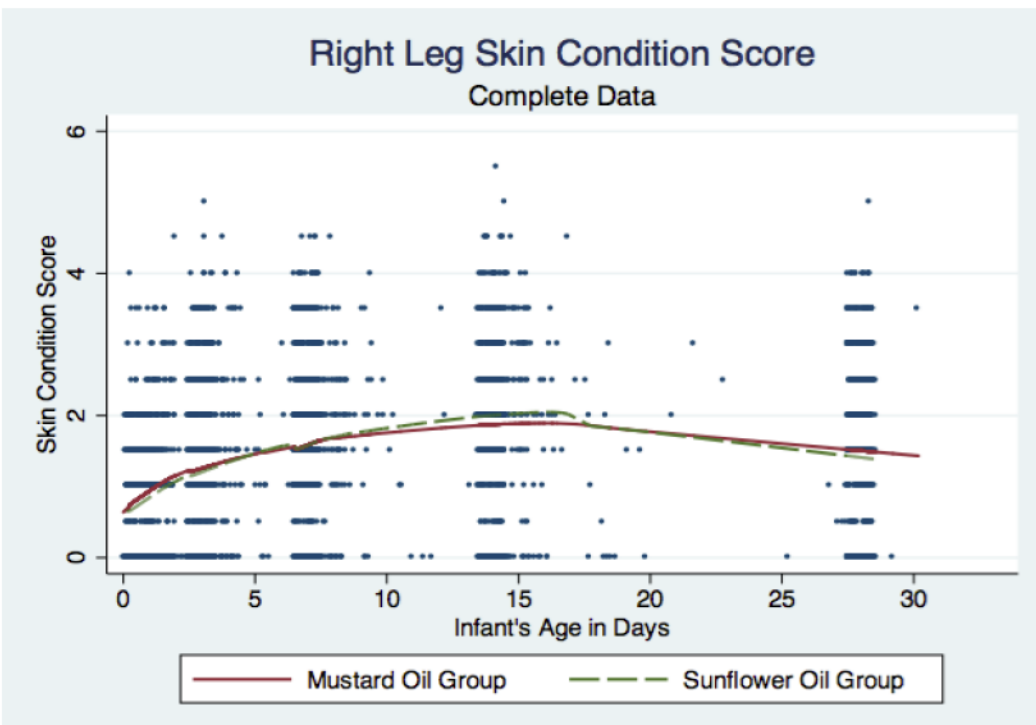


Figure B-11: Right Leg Skin Condition Total Score by Infant's Age-Full Term

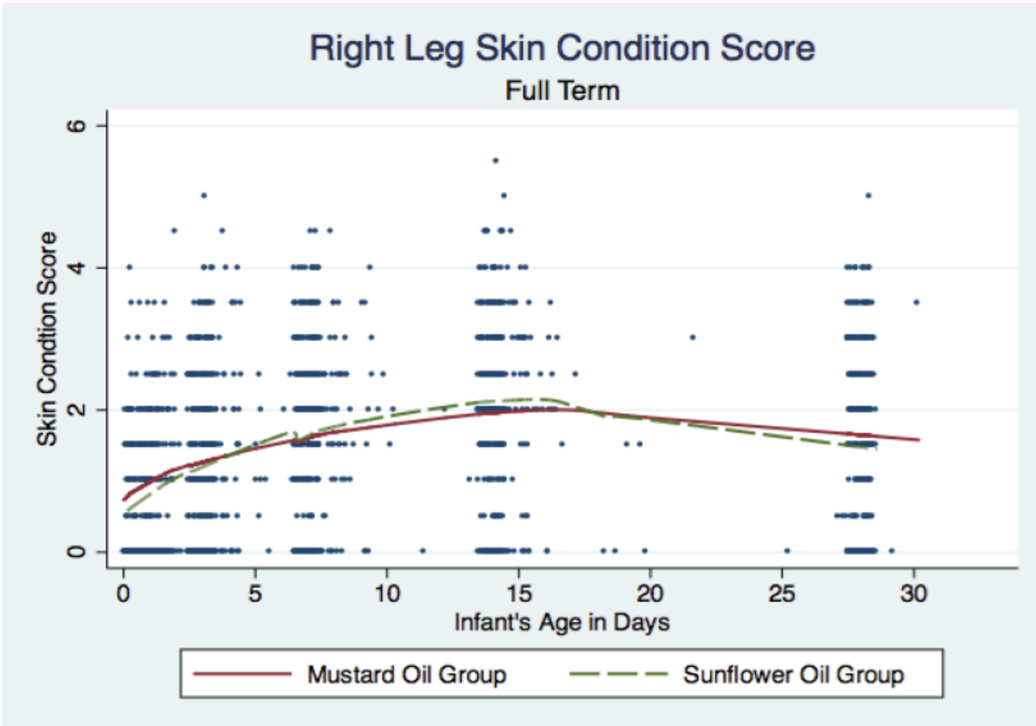


Figure B-12: Right Leg Skin Condition Total Score by Infant's Age-Preterm

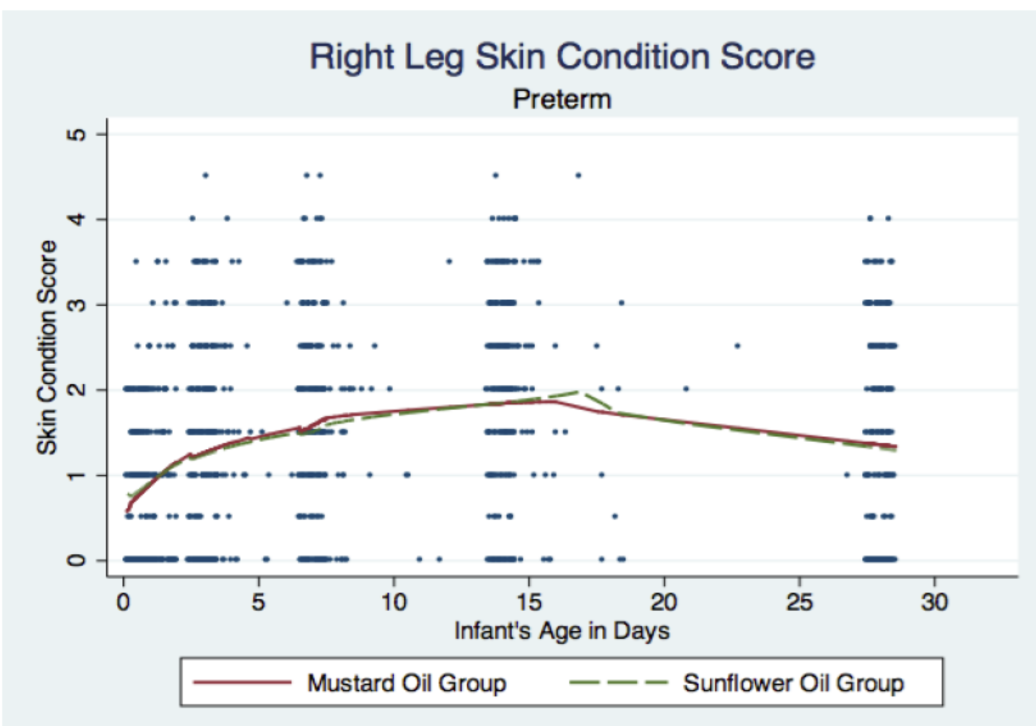
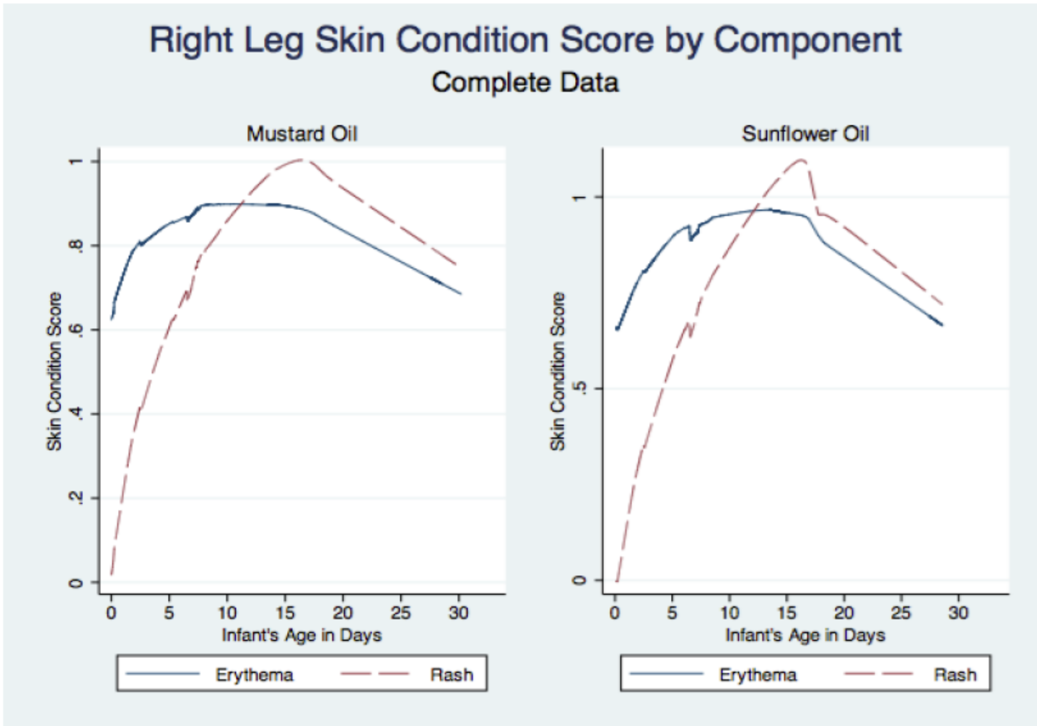


Figure B-13: Right Leg Skin Condition Score by Component and Intervention Group-Complete Data



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Apr 2004 – Aug 2005	**Research Assistant,** Department of Obstetrics and Gynecology, University of Michigan Hospital, Ann Arbor, MI, USA

Honors and Awards

May 2013	**Dan David Prize Scholarship,** Future Time Dimension, Preventative Medicine
May 2013	**R. Bradley Sack Family Scholarship in Infectious Diseases,** Johns Hopkins School of Public Health
May 2013	**International Health Alumni Scholarship,** Johns Hopkins School of Public Health
May 2012	**The David and Elinor Bodian Scholarship Fund,** Johns Hopkins School of Public Health
Jan 2012	**Global Health Conference Grant Recipient,** Johns Hopkins School of Public Health
June 2011	**Global Health Field Placement Award,** Johns Hopkins School of Public Health
May 2011	**Nancy Stephens Fund,** Johns Hopkins School of Public Health
Dec 2010	**National Institute of Health Maternal and Child Health Training Grant,** Johns Hopkins School of Public Health, Dr. Joanne Katz
May 2009	**Delta Omega Honor Society,** Johns Hopkins School of Public Health
May 2006	**Baker, Reinke, Taylor Scholarship in International Health,** Johns Hopkins School of Public Health
Apr 2004	**Magna Cum Laude,** University of Michigan

Publications

Fuller JA*, **Summers A***, Katz MA, Lindblade KA, Njuguna H, Arvelo W, Khagayi S, Emukule G, Linares-Perez N, McCracken J, Nokes J, Ngama M, Kazungu S, Mott JA, Olsen SJ, Widdowson MA, Feikin DR**. *Estimation of the national disease burden of influenza-associated severe acute respiratory illness in Guatemala and Kenya: a novel methodology***. PLoS One. 2013 Feb;8(2).
*=Co-first authors

Summers A, Winkel L, Hussain H, DeLancey JOL. ***The relationship between anterior and apical compartment support***. Am J Obstet Gynecol. 2006 May;194(5):1438-43.

Stein TA, Kaur G, **Summers A**, Larson KA, DeLancey JO. ***Comparison of bony dimensions at the level of the pelvic floor in women with and without pelvic organ prolapse***. Am J Obstet Gynecol. 2009 Mar;200(3):241.

Hsu Y, Chen L, **Summers A**, Ashton-Miller JA, DeLancey JO. ***Anterior vaginal wall length and degree of anterior compartment prolapse seen on dynamic MRI***. Int Urogynecol J. 2008 Jan;19(1):137-42.

Hsu Y, **Summers A**, Hussain HK, Guire KE, DeLancey JO. ***Levator plate angle in women with pelvic organ prolapse compared with women with normal support using dynamic MR imaging***. Am J Obstet Gynecol. 2006 May;194(5):1427-33.

Presentations

"**Methodology to calculate the annual disease burden of influenza-associated severe respiratory illness in Kenya using population-based and sentinel surveillance data**", Third Annual African Network for Influenza Surveillance and Epidemiology (ANISE) Meeting, Nairobi, Kenya, 2012: oral presentation of a methodology developed to estimate the annual disease burden of severe influenza in Kenya.

"Pneumococcal disease in older children and adults globally: results from the AGEDD Project" Cristina R. Garcia, Hope L. Johnson, **Aimee Summers**, Xue Wang, Chou Cheng Lai, Krit Pongpirul, Orin S. Levine, Maria Deloria-Knoll, Katherine L. O'Brien. The 8th International Symposium on Pneumococci and Pneumococcal Diseases, Iguacu Falls, Brazil, 2012: poster presentation on the results of the Adult Global Estimation of Disease Burden and Distribution of Serotypes of Serious Pneumococcal and Meningococcal Disease (AGEDD) project.

"The relationship between anterior and apical compartment support", Annual American Urogynecologic Society Scientific Meeting, Atlanta, GA, 2005: oral presentation of the findings on the correlation between support of the anterior and apical compartments.